EVALUATION OF ADJUVANTS IN FUNGICIDE SPRAY APPLICATION FOR THE CONTROL OF ALTERNARIA BROWN SPOT IN SOUTH AFRICAN CITRUS ORCHARDS

by

JOHANNES GIDEON VAN ZYL

Dissertation presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the Faculty of AgriSciences at the University of Stellenbosch

Supervisor: Prof. Paul H. Fourie

April 2019

The financial assistance of the National Research Foundation (NRF) towards this research is hereby acknowledged. Opinions expressed and conclusions arrived at, are those of the author and are not necessarily to be attributed to the NRF.

DECLARATION

By submitting this thesis/dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: December 2018 Sign: Johannes Gideon van Zyl

SUMMARY

Citrus fruit and foliar diseases are mainly controlled through pre-harvest application of fungicides. Fungicides are only as effective as the application process and for effective disease control deposition of a uniformly distributed quantity of active ingredient(s) is required on the intended target(s). Adjuvants have the potential to improve fungicide deposition on a target surface. The influence of adjuvants on the deposition of fungicides, especially at the high spray volumes used in South African citrus production is unknown and was therefore investigated.

A previously developed deposition assessment protocol, using a yellow fluorescent pigment as tracer for copper oxychloride (CuOCl) deposition, was improved through photomacrography and digital image analyses which proved accurate in determining the quantity and quality of deposition on citrus leaves. Spray deposition benchmarks indicative of the biologically efficacy of CuOCl against *Alternaria alternata* [causal agent of Alternaria brown spot (ABS) of mandarins] was developed.

The deposition assessment protocol and deposition benchmarks was used to evaluate two organosilicone adjuvants (Break-Thru S240 and Break-Thru Union) at reduced spray volumes in dense and less dense citrus canopies in two separate orchard spray trials. Deposition quantity generally increased with increasing spray volume, but normalised values showed better spray efficiency at lower volumes. In pruned and less dense canopies, a beneficial effect of adjuvants was observed in terms of deposition quantity, efficiency and uniformity, especially at reduced volume applications. Some improvement in deposition quality was generally observed with the use of adjuvants. These benefits were not as evident in very dense canopies, illustrating the importance of canopy management when spraying at reduced volumes.

Commercially available adjuvants [Break-Thru, Nu-Film-17, Citrole100, Villa51, Wetcit, Entrée and Exit] were evaluated in three orchard spray trials on different citrus types, cultivars and spray volumes. In trial one, adjuvants improved deposition quantity and canopy penetration. In trial 2 and 3, deposition quantity was generally higher at higher spray volumes, but spray efficiency was significantly better at lower spray volumes. Adjuvants generally improved deposition uniformity and deposition quality, but these benefits were significantly influenced by spray volume and the specific adjuvant treatment. Poor performance by adjuvants was ascribed to high spray volumes and/or too high adjuvant concentration used, which led to increased levels of run-off and poor deposition parameters.

The effects of adjuvants on deposition quantity, quality and biological efficacy of CuOCl against ABS on mandarin leaves were determined in laboratory trials. Adjuvant treatments varied significantly in deposition quantity and quality and disease control achieved. Higher

deposition quantity, beter quality and higher Cu residues was realized at pre- *vs.* post-run-off volumes. Adjuvants did not improve deposition parameters compared with the control treatment at both spray volumes. Leaf infection analysis indicated that CuOCl with adjuvant sprays (post-run-off volume) realized similar and in some cases slightly better control (although not significant) than copper oxychloride alone, but that deposition and Cu residue loading in some of these adjuvant treatments were markedly lower. This anomaly could be ascribed to direct or indirect effects of the adjuvant and was investigated further.

In vivo and *in vitro* studies were done to identify possible direct adjuvant effects on pathogen development and potential synergistic effects between the adjuvants and CuOCl. Adjuvants alone did not influence conidial adhesion, appressorium formation, germ tube length and percent viable conidia. Adjuvant sprays together with CuOCl reduced conidial adhesion, germ tube length and percent viable conidia numerically; however, not significantly compared with CuOCl alone. Adjuvants also caused conidium/germ tube stress similar to CuOCl, but did not inhibit germination or growth. In the *in vitro* microtiter assay, adjuvants together with CuOCl improved germination or growth inhibition compared with the CuOCl treatment alone, although not at significant levels. The findings in Chapter 6 did not fully explain the anomalous findings in Chapter 5, and future studies should focus on developing methodology to support histopathology studies on sensitive leaf surfaces, as well as development of a more sensitive method of measuring deposition quality, especially on a microscopic scale.

OPSOMMING

Sitrus vrug- en blaarsiektes word hoofsaaklik deur voor-oes spuit toediening van swamdoders bestuur. Swamdoders is slegs so effektief soos die spuit toedieningsproses. Vir effektiewe siektebestuur word ‘n homogene verspreiding van die regte kwantiteit aktiewe bestandeel of bestandele verlang op die nodige teiken(s). Byvoegmiddels besit die potensiaal om swamdoder deposisie op verlangde teiken oppervlaktes te verbeter. Die invloed wat byvoegmiddels op deposisie parameters het, veral teen die hoë spuitvolumes wat in Suid-Afrikaanse sitrus produksie gebruik word, is onbekend en was gevolglik ondersoek.

’n Voorheen ontwikkelde deposisie assesseringsprotokol is verbeter deur die gebruik van ’n geel fluoresserende pigment wat dien as ’n “tracer” vir koperoksichloried (CuOCl) deposisie, fotomakrografie en digitale beeld aneliese. Die verbeterde protokol kon deposisie kwantiteit sowel as kwaliteit akkuraat op sitrus blare bepaal. Deposisie drempelwaardes aanduidend van die biologiese effektiwiteit van CuOCl teen *Alternaria alternata* [die veroorsakende organisme van Alternaria bruin vlek (ABS) van mandaryne] is ontwikkel.

Die verbeterde deposisie assesseringsprotokol en drempelwaardes is gevolglik gebruik om twee organosilikoon byvoegmiddels (Break-Thru S240 en Break-Thru Union) by verlaagde spuit volumes in digte en minder digte sitrus lowers in twee aparte boorde te evalueer. In die algemeen het deposisie kwantiteit toegeneem met toename in toedieningsvolume. Genormaliseerde deposisie kwantiteit waardes het beter spuit effektiwiteit uitgewys by laer toedieningsvolumes. Daar was ’n verbetering in deposisie kwaliteit waargeneem met die gebruik van byvoegmiddels. Hierdie voordele was nie so duidelik in digte lowers nie, wat die belangrikheid van lowerbestuur, wanneer verlaagde spuit volumes gebruik word, uitgewys het.

Kommersiëel beskikbare byvoegmiddels [Break-Thru, Nu-Film-17, Citrole100, Villa51, Wetcit, Entrée and Exit] is op verskillende sitrus tipes, kultivars en spuit volumes in vier boord spuitproewe geëvalueer. Beter deposisie kwantiteit sowel as lower penetrasie was verkry deur die gebruik van benatters in proef 1. Deposisie kwantiteit was in die algemeen hoër by hoër spuitvolumes, terwyl deposisie effektiwiteit beduidend beter was by laer spuit volumes in proef 2 en 3. Byvoegmiddels het in die algemeen deposisie uniformiteit sowel as kwaliteit verbeter, maar hierdie voordele was noemenswaardig beïnvloed deur spuitvolume en die spesifieke byvoegmiddel behandeling. Swak resultate met die gebruik van byvoegmiddels is toegeskryf aan hoë spuit volumes en/of te hoë byvoegmiddel konsentrasie wat gebruik is. Hierdie invloede het gelei tot verhoogde afloop en daarvolgens swakker deposisie vlakke.

Die invloed van byvoegmiddels op deposisie kwantiteit, kwaliteit en die biologiese effektiwiteit van CuOCl teen ABS op mandaryn blare is in ’n laboratorium studie geëvalueer. Byvoegmiddel behandelings het betekenisvol verskil kragtens deposisie kwantiteit, kwaliteit en ABS beheer verkry. Hoër deposisie kwantiteit, beter kwaliteit en hoër Cu residuvlakke is

by voor- *vs.* na-afloop spuitvolumes verkry. Byvoegmiddels het nie deposisie vlakke in vergelyking met die kontrole behandeling by beide spuitvolumes verbeter nie. Blaar infeksie analiese het uitgewys dat CuOCl tesame met byvoegmiddel spuite (teen na-afloop spuit volumes) dieselfde en in sekere gevalle beter (alhoewel nie betekenisvol nie) siektebeheer as die CuOCl alleen spuit gelewer het. Tog is waargeneem dat deposisie en Cu residu in sommige gevalle, afhangende van die behandeling, laer was. Hierdie anomalie kan toegeskryf word aan moontlike direkte en indirekte effekte wat byvoegmiddels kan hê en is daarvolgens verder ondersoek.

In vivo en *in vitro* studies is gedoen om moontlike direkte byvoegmiddel effekte op patogeen ontwikkeling sowel as potensiële sinergistiese effekte tussen byvoegmiddels en CuOCl te identifiseer. Byvoegmiddels op hul eie het nie konidiale vashegting, appressorium ontwikkeling, kiembuislengte of die persentasie lewendige konidia noemenswaardig beïnvloed nie. Byvoegmiddel spuite tesame met CuOCl het konidium aanhegting, kiembuis lengte en die persentasie lewendige konidia verlaag, tog nie betekenisvol in vergelyking met die CuOCl alleen spuit nie. Byvoegmiddels het ook konidium/kiembuis stress veroorsaak wat vergelykbaar was met die CuOCl spuit, alhoewel dit nie ontkieming of groei beïnvloed het nie. In die *in vitro* “microtiter” toets het byvoegmiddels tesame met CuOCl ontkieming of groei vertraging gewys in vergelyking met die CuOCl alleen behandeling, maar tog nie betekenisvol nie. Die bevindings in hoofstuk 6 kon nie ten volle die teenstrydige bevindinge van hoofstuk 5 verduidelik nie. Daarom moet toekomstige studies fokus op die ontwikkeling van metodes om histopatologie studies moontlik te maak op sensitiewe blaar oppervlaktes, sowel as die ontwikkeling van meer sensitiewe metodes om deposisie kwaliteit te meet, veral op ’n mikroskopiese skaal.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and appreciation to the following persons and institutions:

Prof Paul H Fourie, my mentor, advisor and promotor to whom I would like to express my sincerest gratitude for his guidance, knowledge, wisdom, motivation and unfailing continuous support and patience through the process of researching and writing of this thesis. His guidance in research, science and life made me the person I am today. Prof Fourie provided me with the opportunity that led to this study and the person I am today. I could not have imagined having a better advisor and mentor. I am forever grateful to him.

Janine van Zyl, my loving wife, always encouraging me to do and be better through all her support, patience and love.

My family, providing me with the opportunity financially and for all the encouragement and support.

Citrus Research International for providing me with the opportunity and support, financially and academically.

The department of Plant Pathology students and staff for providing me with facilities, support and being the best colleagues and friends I could ever have. It is an honour to be part of such a great family of pathologists.

Marieta Van Der Rjist for statistical guidance and analyses.

Spray machine suppliers and farmers for providing machinery and trial sites.

The National Research Foundation, THRIP and Bayer CropScience South Africa for financial support.

Our heavenly Father for his mysterious ways. Even though I do not understand Him and His ways, I am grateful.

CONTENTS

CHAPTER 1

An overview of spray adjuvant use in fungicide spray application for the control of fungal diseases in citrus

INTRODUCTION

South Africa is the 13th largest producer of citrus in the world, producing annually over 2,231,000 tons of citrus from 77,708 ha of citrus plantings; 1,692,000 tons of the total produce is exported annually as fresh market citrus (76% of total production), ranking South Africa as the third largest exporter of fresh market citrus world-wide, accessing more markets than any other citrus producing country – positioning citrus production as a major role player in the South African economy (CGA Key industry statistics 2018).

Citrus production and access to export markets are threatened by fruit and foliar diseases such as Alternaria brown spot (ABS) (*Alternaria alternata* (Fr: Fr) Keissl., tangerine pathotype) (Schutte, 1996; Timmer, 2000; Timmer *et al*., 2000), citrus black spot (CBS) (*Guignardia citricarpa* Kiely) (Schutte *et al*., 1997; Kotzé, 2000), and melanose (*Phomopsis citri* H. Fawcett non (Sacc.) Traverso and Spessa) (Whiteside and Timmer, 2000). If not controlled adequately, these diseases can cause major economic losses and curb exports.

In South Africa and most other citrus-producing countries, fruit and foliar diseases are mainly controlled through the use of fungicides. However, these fungicides are only as effective as their application, timing of application and the sensitivity of the pathogen population to the fungicide(s) used. Therefore, the main objective of fungicide spray application is the optimal transfer of the correct dose of a well agitated fungicide tank mixture, which may include one or more active ingredients that are compatible, added and mixed in the correct order; from the spray applicator that is using the correct nozzle selection depended on the spray volume and tree characteristics, to the tree, whilst keeping off-target losses from run-off and drift to a minimum. For effective disease control, deposition of a uniform distribution of the required quantity of active ingredient(s) (optimal dose transfer) is required on the intended targets. This must be achieved whilst balancing an optimal equilibrium between efficacy and efficiency based on present economic conditions, which in reality is a complex task.

As the trend is worldwide (Stover *et al*., 2002; 2003), spray application methodology and technology used by citrus growers in South Africa is predominantly influenced by the most important economical diseases, export and/or quarantine regulations/restrictions and the fear and reality of losing disease control. Therefore, South African producers rely heavily on medium to high volume fungicidal spray applications (6000 to 10 000 L ha^{-1}) (Grout, 1997; 2003). This is higher than spray volumes used in other citrus producing countries, for example

Spain (1000 to 2500 L ha^{-1}) (Garcerá *et al*., 2011; 2014; 2017) and the United States of America (200 to 4500 L ha^{-1}, in some cases up to 7000 L ha^{-1}) (Stover and Salvatore, 2002; Salyani and Farooq, 2005; Salyani *et al*., 2007; Salyani, 2015) to secure market access and protect citrus fruit from infection.

Fungicide sprays are repeated 4 to 5 times (every +/-21 to 35 days) a season, depending on spore release events and the fungicides used, to ensure adequate coverage of rapidly expanding fruit and new flush growth and product/coverage weathering loss due to rainfall (Schutte *et al*., 2012; 2014; Kotzé *et al*., 2018). These application volumes do provide an acceptable balance between efficacy and efficiency based on existing economic considerations. Most importantly, it serves as a "buffer" against poor application, due to calibration and operator error and/or the use of inadequate spray machinery, equipment and technique. However, high spray volumes run the risk of loss of efficacy/effectiveness due to spray run-off and exo- and endo-drift (Salyani and Farooq, 2005; Fourie *et al*., 2009; Cunha *et al*., 2012; Schutte *et al*., 2012). Off-target deposition of fungicides is increased at excessive high spray volumes (8000 L ha^{-1} and higher) (van Zyl and Fourie, unpublished results), which in turn is an economical loss and an environmental pollution problem (Stover *et al*., 2002; Meli *et al*., 2003; Salyani and Farooq, 2005; Furness *et al*., 2006a, 2006b; de Jong *et al*., 2008; Cunha *et al*., 2012; Gregorio *et al*., 2016).

Spray application is influenced by a mass of contributing factors, making it a difficult field of study. However, understanding how these factors influence spray application and therefore deposition and retention, will lead to the improvement and better implementation of fungicide application and therefore improved disease control. Various methodologies for the evaluation of spray deposition effectiveness have been developed for a range of crops. Methods of evaluation range from relatively simple to more advanced methods. These include qualitative visual assessment of spray deposition on sprayed targets through the use of fluorescent tracers (Salyani and McCoy, 1989; Holownicki *et al*., 2002; Furness *et al*., 2006a; 2006b) and the use of droplet rating charts to evaluate deposition on actual or artificial targets (Holownicki *et al*., 2002; Furness *et al*., 2006a). These methods are relatively simple but lack the ability to accurately measure deposition quantity and quality since it is dependent on human discretion (Salyani and Whitney, 1988; Jiang and Derksen, 1995). More advanced methods for determining deposition quantity include chemical residue recovery techniques such as gas chromatography or atomic absorption, spectrophotometry of metals and nutrients (Ware *et al*., 1969; Yates *et al*., 1974; Byers *et al*., 1984) and also recovering sprayed fluorescent tracers from artificial and plant surfaces through washing techniques and determining deposition through fluorometry and colorimetry (Lake, 1988; Salyani and Whitney, 1988; 1990). These methods lack the ability to quantify the quality of coverage, such as uniformity of spray coverage on the target surface (Juste *et al*., 1990). Spray deposition measurement,

specifically in terms of quantity and quality, was improved through the development of deposition assessment protocols that combines fluorometry, digital photomicrographic imaging and digital image analysis (Hoffmann and Salyani, 1996; Brink *et al*., 2004; 2006; Fourie *et al*., 2009; van Zyl *et al*., 2010a; 2010b). In their work, they assessed spray deposition and the effect it has on disease control by the following parameters:

- **Deposition quantity –** The amount (quantity) of active ingredient(s) available on the target surface/site to protectagainst disease.
- **Deposition quality –** The uniformity/distribution of active ingredient(s) deposition/retention on the target site/surface.
- **Deposition uniformity –** The uniformity of active ingredient(s) deposition between target sites on a target organism (multiple leaves, fruit and twigs – target dependent).

Above-mentioned factors are all influenced by the canopy geometry and density of the target (Cross *et al*., 2001a; 2001b; Jejčič *et al*., 2011; van Zyl *et al*., 2014), environmental conditions (Salyani, 2005; 2006), the use of appropriate machinery and equipment (Cooke and Hislop, 1993; Cunningham and Harden, 1998a; 1998b; 1999; Furness *et al*., 2006b; Salyani, 2005; 2006; Nuyttens *et al*., 2007; Zwertvaeger *et al*., 2014), spray technique and calibration method used (Salyani and Whitney, 1990; Furness *et al*., 1998; Cross *et al*., 2001a, 2001b, 2001c; Salyani and Farooq, 2005), spray volume (Salyani and Hoffmann, 1996; Cunningham and Harden, 1999; Fourie *et al*., 2009), the fungicide or pesticide used (Sundaram and Sundaram, 1987; Zabkiewicz, 2007), the influence of adjuvants (Butler-Ellis *et al*., 1997; Gent *et al*., 2003; Green and Beestman, 2007; van Zyl *et al*., 2010a; 2010b), and the complex interaction between these factors (Whitney *et al*., 1988; 1989; Cross *et al*., 2001a, 2001b, 2001c; Grout, 2003; Salyani, 1994; 2005; 2006; Stover *et al*., 2002).

Adjuvants are regularly used in fungicide sprays in South African citrus orchards. It has many functions. With fungicide sprays, it mostly acts as a product to stabilise the spray mixtures pH, reduce foaming and improve deposition parameters of the fungicide spray through wetting, spreading and sticking (Hazen, 2000; Hock, 1998; Tu and Randall, 2003). There are various different formulations of adjuvants available on the market for use with citrus sprays. In South Africa, there were currently 14 registered adjuvant products for use in citrus production in 2018 (www.agri-intel.com). Research on adjuvant use with fungicide sprays and the effect it has on deposition parameters and disease control is very limited (Steurbaut, 1993; Stevens, 1993). Furthermore, research into adjuvant use in citrus production is almost non-existent. This makes it very hard for the user to make an informed decision of which adjuvant, and at which rates, to use depending on the scenario. The following chapter will give an overview on the focus of this study: Spray application in South African citrus production, methods of measuring and evaluating spray deposition parameters, the use of adjuvants in

fungicide spray application, and an overview of the model pathogen used in this study, *A. alternata*, the causal agent of ABS of mandarins.

"A pesticide can be expected to be effective if the 'right material' is applied, at the 'right amount', on the 'right target', at the 'right time', with the 'right sprayer' under the 'right weather' conditions".

M. Salyani

Spray application in South African citrus production

As with most other three-dimensional crops (stone fruit, pome fruit and grape production etc.) in South Africa, spray application of fungicides, pesticides, foliar feeds and growth regulators in citrus production are applied with the use of tractor drawn and driven air-assisted or hydraulically pressurised sprayers. These sprayers or applicators are power take-off (PTO) driven, which powers either an axial or centrifugal fan to create an air column with a certain volume (m h^{-3}) at a certain velocity (m s^{-1}) with a specific air profile (Landers, 2010). Axial fans draw air from either the front or the back and project it 90˚ into the housing towards the nozzles. With centrifugal fans the air is drawn into the centre of rotating blades, which redirects it at 90˚. Velocity and volume of air depend on the number, size (diameter), curvature and pitch (degrees) of the fan blades, whilst the profile depends on the shape of the housing of the fan. This housing can be short, as in the case of low-profile sprayers, or tall, as in the case of high-profile sprayers or tower sprayers (Landers, 2010; Personal communication, Marius Ras, Rovic and Leers, South Africa). Axial fans usually have a high air volume to low velocity ratio, whilst centrifugal fans have the opposite ratio. Flow/profile can furthermore be manipulated by adjustable or non-adjustable deflectors. The air column is directed at the fruit tree (target) and is loaded with droplets of a certain size spectra that is created by spray nozzles in the case of axial fan sprayers, or spray liquid that is sheared into droplets by air as in the case of centrifugal sprayers. The droplets are carried in the air column from the sprayer to the tree, where the droplets are deposited on tree structures (targets) that need to be protected from insect damage and fungal or bacterial infection. In the case of hydraulic pressure sprayers, the spray liquid is sheared through a nozzle by pressure, with the created droplet "shot" to the target (tree) without any air assistance (Landers, 2010; Personal communication, Marius Ras, Rovic and Leers, South Africa).

Spray volumes used in South African citrus production are high compared to other countries. Producers use medium to high volume fungicide and pesticide spray applications (6000 to 10 000 L ha^{-1}) (Grout, 1997; 2003). This is markedly higher than spray volumes used in other citrus producing countries, for example Spain (1000 to 2500 L ha^{-1}) (Garcerá *et al*., 2011; 2014; 2017) and the United States of America (200 to 4500 L ha^{-1}, in some cases up to

7000 L ha^{-1}) (Stover and Salvatore, 2002; Salyani and Farooq, 2006; Salyani *et al*., 2007; Salyani, 2015). It is plausible that higher spray volumes for fungal disease control has largely evolved unintentionally from control methods developed for the control of Californian red scale (*Aonidiella aurantii* (Maskell)) through medium to high volume mineral/petroleum-based oil, pesticide and oil combination sprays using hand lances following to the development of organophosphate pesticide resistance in the 1970's in South Africa (Georgala, 1975). Additionally, as was the case in Florida citrus production in the United States of America (Cromwell, 1975), the transition from pressurised hand lance sprays to tractor drawn spray machines in the 1940s and 50s also resulted in growers trying to duplicate the same degree of 'wetting' or 'cover' with the tractor drawn machines as was obtained with hand lances, even though the modern sprayers are capable of producing effective spray plumes with various nozzle and fan technologies at lower spray application volumes (Personal communication, Tim Grout, Citrus Research International, Nelspruit, South Africa). High spray volume use can also be ascribed to growers believing that citrus trees being large and dense, with its geometry complicating adequate spray deposition and penetration (Larbi and Salyani, 2012), need very high spray volumes to achieve adequate deposition and penetration.

This present trend of application do provide disease control and has an acceptable impact on production cost, but is becoming more uneconomical year by year. Furthermore, it gives producers "peace of mind", especially in the case of controlling CBS given its quarantine status in certain export markets (EPPO, 2014). This is because it serves as a "buffer" for loss of efficacy due to calibration and operator error and the use of inadequate machinery, equipment and technique.

Grout (1997) suggested a simple method to determine the spray volume needed depending on the type of spray application. The methodology was partly based on the Tree-Row-Volume concept developed by Byers (1987) and Sutton and Unrath (1984, 1988). The Tree-Row-Volume concept is based on the theory that a row of fruit trees has a certain volume of plant material that can be calculated taking the tree height, tree depth and row width into account. For each m^3 of plant material per ha, a certain spray volume would be needed to "wet" the material to the point of run-off. Depending on the density of the plant material, the amount of spray liquid needed would vary (Unrath, 2002). However, since most citrus rows do not form a rectangular box due to the tree's spherical form, TRV calculations were mostly super optimal when calculated for citrus trees. Grout's (1997, 2003) simpler method assumed that modern citrus orchards forms a hedge-row and that if there were to be gaps between rows, producers still spray the gaps (basically gaps are ignored). His method takes tree height and row length into account and depending on the type of application and the density of the trees in the orchard, sets out a table to refer to the amount of liters per row length that is

needed for adequate spray deposition. Variable factors therefore are tree height, density and the number of rows per block.

Unfortunately, this system is rarely used with producers usually using fixed spray volumes depending on the type of application needed. These are commonly revered to in South Africa as outside cover (outer tree canopy only) application, medium cover (outer canopy and branches up to a diameter of 75 mm) and full cover (all plant parts above ground) application and are regularly seen on plant protection product labels registered for use in citrus production in South Africa. Spray type is usually target specific (volume based on where the target is situated). Outside cover spray applications are used for thrip sprays, thrip bait sprays, foliar feed applications, and light bollworm, leafroller and orange dog applications. Medium cover spray applications are used for applications against false codling moth, fungal diseases, miticide sprays, looper, leafhopper and stinkbugs. Full cover spray application is used for red scale, mealybugs etc. Even though these guidelines are well set out and freely available, most producers use a low volume application (usually half the spray volume of the high-volume application, which is achieved by decreasing tractor speed rudimentary by using one lower forward gear speed) for outside coversprays and a high-volume application (full cover) for all other insecticidal and fungicidal applications (Grout 1997, 2003).

All plant protection products (PPP) for application in citrus production in South Africa are registered as a concentration (expressed as rate per 100 L) or as the maximum allowed dose of PPP per ha by regulation of Act 36 of 1947. Most PPPs, if not all, is registered to be applied as high-volume sprays to ensure full coverage of foliage and stems and is worded as such. Thus, using any spray volume besides a high-volume application at the recommended concentration, or a reduced volume with concentration amended to the spray volume would be contradicting the act. These registered label recommendations prohibit producers from using lower application volumes.

High volume dilute sprays are in most cases super-optimal. It is costly and not efficient in terms of time and input costs. Furthermore, it increases spray run-off, exo- and endo-drift (Salyani *et al*., 2007). This increase in off-target deposition is an unnecessary economical loss and a potential environmental problem (Vercruyesse *et al*., 1999; Cunha *et al*., 2012). Spray run-off, as influenced by various factors in different cropping systems (Salyani and Whitney, 1990; Cunningham and Harden 1998; 1999; Furness *et al*., 1998; Farooq and Salyani, 2002; Stover *et al*., 2002; Salyani *et al*., 2007; Chueca *et al*., 2009; Cross *et al*., 2011), particularly high volume sprays, which is a well-documented phenomena but often ignored since high volume sprays act as a buffer for other shortcomings during the application process, such as mistakes in calibration, technique, or equipment and operator error. The redundancy of this style of application needs to be addressed.

The influence of spray machine type and calibration on deposition parameters have been studied in citrus production to great extent (Cunningham and Harden, 1999; Farooq and Salyani, 2002; Khot *et al*., 2012; Pai *et al*., 2009) with the main aim of improving deposition and reducing product losses through various methods of optimisation. Citrus trees are complex targets due to high variation in height, width, depth, shape (canopy volume) and foliage density. Citrus tree canopy volume and foliage density vary from citrus type, cultivar, rootstock selection and climatic region. Large variations can be found between trees even in one orchard of the same citrus type and/or cultivar (Whitney *et al.,* 1999). Most spray calibration (spray volume determination, spray speed etc.) are made on the assumption that orchard canopies are uniform. This is mostly super optimal due to the high variation in tree geometry, volume and density that can be present in one orchard and between orchards. Furthermore, orchards differ in plant and row spacing, further complicating the use of a single calibration setup. Thus, one calibration for a farming unit with various orchards would not be optimal for all trees and will result in over or under application, drift and environmental pollution.

Ideally one would set up sprayer calibration for a specific orchard. Accurate measurement of canopy geometry and density is needed for this step. Various manual measurement protocols in citrus have been developed and evaluated (Albrigo *et al.,* 1995; Wheaton *et al*., 1995). Manual measurement of trees can be laborious and time consuming, and cannot be done for every tree in an orchard. The development of electronic measurement systems including ultrasonic sensors (Tumbo *et al*., 2002; Zamahn and Salyani, 2004; Solanelles *et al*., 2006) and LIDAR systems (light detection and ranging) (Wei and Salyani, 2004; 2005; Rosell *et al*., 2009) allows for real-time measurement of tree canopies that are non-destructive.

The higher, deeper and denser a canopy is, the more complex it is. The more complex the canopy is, the harder it is to realise uniform deposition on all targets of the tree canopy (top, middle and bottom, inner and outer canopy targets). All spray application is done from the exterior of the tree towards the target. Higher and deeper canopies increase droplet travel time. Thus, as canopy depth increase, deposition parameters, quantity, uniformity and quality, will decrease. Whilst evaluating the influence of two different organosilicone adjuvants on different citrus canopy densities, Van Zyl *et al.* (2014) found that higher deposition quantity on outer canopy leaves than on inner canopy leaves and that less dense canopies were easier to penetrate. Farooq and Salyani (2002) found similar results whilst evaluating different sprayer types in citrus canopies, with deposition decreasing as canopy depth increased. Cunningham and Harden (1999) evaluated various sprayers to reduce spray volumes in mature citrus trees. Two of the four sprayers realised higher deposition on the outer than on the inner canopy. Salyani and Whitney (1990) and van Zyl *et al*. (2014) also found higher variation in deposition quality as canopies became more complex.

Adjuvant classification

The word *Adjuvant* is derived from the Latin word *adjuvare*, which means “to help”. In spray application technology and methodology, adjuvants offer the potential to improve deposition parameters and if possible, uptake of plant protection products on/by the plant surface (Green and Beestman, 2000). An adjuvant is any substance or material that is added to a spray mixture to influence the biological efficacy function of the active ingredient in the spray mixture or by modifying the physical properties of the spray mixture (Hazen, 2000; ASTM, 2016).

There is no formal system to classify adjuvants. Various authors have suggested different classification methodologies for adjuvants to help with selection for specific use. Some suggestions have been to classify adjuvants by mode of action (Kirkwood, 1993) or by where (site) the adjuvant is active (Stock and Holloway, 1993). Stock and Briggs (2000) suggested an adjuvant classification system based on chemical composition to help select adjuvant actives and combinations with specific physiochemical properties. The most common classification system used today was suggested by Hazen (2000) and further described by McMullan (2000) and Penner (2000). Hazen (2000) classified adjuvants into two groups depending on the function or action of the adjuvant: adjuvants that influence the physical properties of the spray solution, and adjuvants that influence the biological efficacy of the agrochemical in solution. The author classified it as “modifier or utility” adjuvants, or “activator” adjuvants, respectively. The precise composition of adjuvant formulations must be known to be able to classify their properties. Some formulations might have more than one active ingredient pertaining to more than one property, thereby classing the composition into various classes (Stock and Briggs, 2000).

Utility adjuvants

Utility adjuvants influence the physical and chemical properties of the spray solution. Through this, it indirectly influences the performance of the pesticide used. Depending on the type of utility adjuvant used, the spray mixture is modified by improving compatibility of two or more incompatible agrochemicals in the tank, defoaming the tank mixture, improving drift control of the agrochemical sprayed, water conditioning, acidifying, buffering or colouring the mixture (McMullan, 2000).

Compatibility agent

A compatibility agent is defined by the American Society for Testing and Materials (ASTM) as “a surface-active material that allows simultaneous application of liquid fertilizer and agrichemical, or two or more agrichemical formulations, as a uniform tank mix, or improves homogeneity of the mixture and uniformity of application” (ASTM, 2016). Compatibility agents

consists out of phosphate esters and anionic surfactants to form a homogeneous spray mixture by dispersing incompatible fertilizers and/or agrochemicals that might have formed a non-homogenous mixture that is not sprayable (McMullan 2000).

Defoaming agent

The ATSM (2016) defines a defoaming agent as “a material that eliminates or suppresses foam in the spray tank”. A foaming tank due to fertilizer or agrochemical content is undesirable since it prohibits the tank to be filled properly, and foams out of all openings, which can lead to contamination of the environment and the operator, and after spraying can make cleaning the tank laborious and wasteful after spraying. Foam is caused by surfactants, agrochemicals or fertilizers that reduces surface tension to a level that allows air to enter the mixture. Foaming can be reduced by adding a defoaming agent that reduces the surface tension even further, by adding silica containing defoaming agents that physically bursts the bubbles, or by adding an oil that changes the foam structure (McMullan, 2000).

Drift control agent

A drift control agent is “a material used in liquid spray mixtures to reduce spray drift” (ATSM, 2016). The amount of spray drift is a function of prevailing weather conditions during spraying (Nuyttens *et al*., 2006), the formulation of the spray mixture (Butler Ellis and Tuck, 1999; Butler Ellis and Bradley, 2002) and nozzle selection and droplet size (Derksen *et al*., 2007; Nuyttens *et al*., 2009). Drift control agents increases the extensional viscosity of the spray solution, which decreases the shear viscosity, which leads to the formation of coarser droplets. Coarser droplets (above 150 µm) are less prone to drift (McMullan, 2000).

Deposition agent

This is “a material that improves the ability of pesticide sprays to deposit on target surfaces” as described by the ASTM (2016). Pesticide deposition is improved by altering the spray mixture to improve the deposition quantity and quality on the target surface. This is achieved by various activator adjuvants by reducing droplet bounce, refraction and contact angle (Hazen, 2000; McMullan, 2000; Penner, 2000).

Water conditioning agent

These are agents that negates the interaction of ions to improve pesticide efficacy. These are usually sequestering or chelating products, which remove certain ions in solution to prevent it from interacting with the herbicide or pesticide in the mixture (ATSM, 2016; Mcmullan, 2000).

Acidifying and buffering agents

Strong acids in dilute form added to spray mixtures to reduce the pH or compounds added to resist change in pH. By acidifying or buffering a spray mixture, it can improve the working of agrochemical, usually by reducing the speed or tempo of chemical breakdown found in alkaline mixtures, thereby increasing the lifespan of the product. The breakdown is usually by means of alkaline hydrolysis (ASTM, 2016; McMullan, 2000).

Activator adjuvants

These adjuvants are known as surface active agents or in short, surfactants. This group usually has one or more of the modes of action and is well described in Penner (2000). Activator adjuvants are products that help overcome deposition and uptake difficulties posed by the epicuticular wax layer of plant surfaces (Baker *et al*., 1975). Activator adjuvants are classified as follows:

Wetter/spreader

Products that lower the surface tension of the spray mixture on the target surface. This increases the contact angle of the droplet with the target surface. The droplet now spans over a larger area, improving coverage. At very low tension levels, droplets can begin to spread, running into other droplets, which inevitably can form a very thin layer over the target surface. This thin layer might be prone to dry faster, which can be positive for contact deposition, but negative for systemic products, since uptake rate would be reduced. This depends on the critical micelle concentration (CMC). A high CMC is needed to achieve low equilibrium surface tension (EST), which improves spreading but can also lead to run-off. This is typical at high spray volumes together with high adjuvant concentrations (Hazen, 2000; Penner, 2000). Contrary to stated above, various studies have shown improved absorbsion of systemic funigicdes. Gent *et al*. (2003) found a 30% increase of azoxystrobin absorbsion on onions and a 21% absorbsion increase on potatoes with the addition of organosilicone/methelyated seed oil-based adjuvant to sprays.

Stickers

Stickers increases the duration that a product is present on the surface by helping it adhere better. Better adhesion helps to resist weathering and wash-off. These are usually polymeric compounds (Hazen, 2000; Penner, 2000). Interestingly, the function and effectiveness of stickers is questioned. Rossouw *et al*. (2018) included Nu-Film-P in a rainfastness study of mancozeb formulations on apple leaves and found that Nu-Film-P did not improve rain fastness of mancozeb on the apple leaf surface compared with mancozeb alone. However,

Gent *et al.* (2003) found a 41% azoxystrobin absorbsion increase on onions and a 39% increase on dry bean with the addition of a wetter/sticker adjuvant combination to sprays.

Humectants

Humectants decrease the rate at which droplets dry on the surface of the target, improving bioavailability for uptake into the leaf or fruit. Humectants draw moisture form the atmosphere or increases the liquidity to be able to increase lifespan of droplets (Hazen, 2000).

Penetrants

Penetrants are products that softens, dissolve or plasticise the cuticle wax layer to help the agrochemical diffuse through the layer into the epidermal layer from the surface of the leaf or fruit (Hazen, 2000; Penner, 2000). These are commonly used in combination with herbicides.

Film forming polymers

Film forming polymers (FFPs) is an example of an adjuvant with mutliple properties and have therefore multiple classifications. Depending on the composition of the film forming polymer, functions are primarily to increase sticking and spreading, and after deposition reduce weathering of the sprayed product, classifiying it as an activator adjunvant. It can also be used to form a film or barrier over plant material to reduce water loss (Gale and Hagan, 1996). Various studies have also evaluated the possibility of using films or barriers produced by FFPs as a substitude to fungicides for the control of various pathogens. The film creates a physical barrier preventing direct penetration of the pathogen through the cuticle and epidermal layer and also infection through stomatal openings (Walters, 2006). Various studies have evaluated this phenomenon on various pathogen-plant interactions, for example for the control of apple scab on apple leaves and fruit (Percival and Boyle, 2009) and the control of *Botrytis cinerea* on various crops (Elad *et al.*, 1990) using FFPs.

Adjuvant use in spray application to improve fungicide deposition

Adjuvants can provide citrus growers with a powerful tool to optimise spray application through improved spray deposition of the active ingredient on the target surface (de Ruiter *et al.*, 1990; Holloway *et al.*, 2000; Gent *et al.*, 2003; van Zyl *et al.*, 2014), if used correctly. Adjuvants added to spray mixtures influence the surface tension of spray droplets at the air-liquid interface and on the contact angle of the liquid-plant interface, mostly by lowering both. Thus, droplets are less prone to shattering, deflection and bouncing on impact with the leaf surface, reducing off-target losses and improving deposition, especially on hard-to-wet (hydrophobic) targets (Dorr *et al.*, 2015; Mayo *et al.*, 2015).

Published research on the physical, chemical or synergistic effects of adjuvants on the bio-efficacy of fungicides used in citrus is almost non-existing. Physical effects might be

ascribed to the alteration of the plant cuticle by the adjuvant. The cuticle layer of each plant species is unique and it plays a major role in biotic interactions, like pathogen recognition (Craver and Gurr, 2006). Adjuvants can disturb the physical structure of the cuticle layer (Knoche *et al*., 1992; Zabkiewicz, 2007), apart from influencing the amount of active ingredient deposited and retained before and after spray run-off. Adjuvants are known to physically influence surface microstructures such as cuticular foldings and epicultricular waxes that minimise contact area between the spray droplet and the target surface (Wagner *et al*., 2003; Bargel *et al*., 2006) to increase deposition and/or retention (Hall *et al*., 1998). These physical changes to the cuticle may also influence the ability of the pathogen to recognise the host and/or disrupt attachment (Tucker and Talbot, 2001; Carver and Gurr, 2006).

Adjuvants can also have a synergistic or potentiating effect on the fungicide. For example, if an adjuvant reduces the pH of the CuOCl solution, the solubility of copper increases and so does the release of copper ions. Higher amounts of released copper ions can theoretically increase the efficacy against pathogen cells. Abbott (2016) found that certain adjuvants acidified the spray mixture, potentially improving the working of captan and reducing alkaline hydrolysis. Grayson *et al.* (1996a) evaluated the effect of adjuvants on the curative effect of dimethomorph in controlling downy mildew on grapevine leaves in a greenhouse study and found no fungicidal effect of an alcohol ethoxylate, an emulsifiable paraffinic oil and a vegetable oil adjuvant solution evaluated. However, disease control was improved when adding these adjuvants to dimethomorph sprays (Grayson *et al*., 1996a, 1996b). Dimethomorph has a translaminar systemic action and was applied in both studies as a curative spray.

Research on grapevine (van Zyl *et al*., 2010a; 2010b) has shown the potential of adjuvants to improve deposition quantity and quality, as well as disease control. However, spray applications using the extremes of recommended concentrations of certain adjuvants, or set concentrations at different spray volumes, realised significantly different results (van Zyl *et al*., 2010b), indicating the need for more specific recommendations for each crop and application.

On avocados (Gaskin *et al*., 2004; 2008) and kiwis (Gaskin *et al*., 2006), the ability of adjuvants to reduce spray volumes and off-target drift has been demonstrated. The ability of adjuvants to improve retention (rain-fastness) of fungicide sprays with sticking agents on cabbage and bean has also been shown (Gaskin and Steele, 2009). However, contrary results were reported by Rossouw *et al.* (2018) who found that Nu-Film-P, a sticker-spreader adjuvant, did not improve rainfastness of mancozeb on apple leaves. Decaro *et al.* (2016) studied pesticide and fungicide rainfastness when sprayed with and without adjuvants on citrus seedlings following different intervals of artificial rain. Sprays with copper hydroxide and copper oxychloride with and without wetter/sticker adjuvants (polydimethylsiloxane and

phosphatidylcholine), resulted in similar deposits per cm^2 leaf surface and the adjuvants did not improve rainfastness in relation to fungicide sprays alone after 1, 6, 12 and 24 h artificial rain. Van Zyl *et al*. (2014) demonstrated that the effective use of adjuvants in citrus orchards has the potential to improve deposition parameters at reduced spray volumes. Deposition quality and uniformity on leaves with two organosilicone formulations were improved at lower application volumes than the norm; however, these benefits were not as evident in very dense canopies, illustrating the importance of canopy management when spraying at reduced volumes.

Methods for measuring and evaluating spray deposition

Measuring spray deposition parameters (the amount and distribution of product retained on the target surface after application) is a necessity when evaluating and optimising factors that influence spray application. It serves as an indicator if a target is adequately covered for effective disease control with a specific product. More importantly, it is used as a tool to identify what and how spray application factors influence spray deposition.

Different methodologies exist for determining deposition parameters on target organisms. These methodologies can range from simple to complex, inexpensive to expensive, versatile (e.g. able to determine deposition quantity, qualitaty and spatially) to simple (e.g. only quantity), and target destructive to non-destructive type of measurements.

Interest in measurement of deposition parameters started as early as the 1950's, with the assessment being done with the addition of fluorescent dyes and pigment to spray mixtures. Spray deposits would then be visually assessed on the target surface (Staniland, 1959). In 1969, Turner Fluorometers was used to measure fluorescent spray deposits on very small upper and lower leaf areas of various crops (1.12 and 0.81 mm) and was compared with chemical leaf washings. Excellent correlations was found between the two methods. Flaws encountered in their methodology were sample size and analysis time. The Turner Fluorometer could only measure very small parts of a target surface and not whole leaf surfaces. To analyse 7 to 10 leaves was very laborious and too small a sample size. Chemical washing accuracy depended on the quality of work and product used. Furthermore, leaf autofluorescence was a problem and hard to evade. The method was, however, useful at its time and was certainly a step in the right direction (Byass, 1969).

Various methodologies for the evaluation of spray deposition effectiveness evolved on a range of crops. Qualitative visual assessment of spray deposition on sprayed targets through the use of fluorescent tracers (Salyani and McCoy, 1989; Holownicki *et al*., 2002; Furness *et al*., 2006a; 2006b) and the use of droplet rating charts to evaluate deposition on actual or artificial targets (Holownicki *et al*., 2002; Furness *et al*., 2006a) was commonly used. These methods are relatively simple but lack the ability to accurately measure deposition quantity

and quality since it is dependent on human discretion (Salyani and Whitney, 1988; Jiang and Derksen, 1995).

More advanced methods for determining deposition quantity include chemical residue recovery techniques such as gas chromatography or atomic absorption, spectrophotometry of metals and nutrients (Ware *et al*., 1969; Yates *et al*., 1974; Byers *et al*., 1984), and also recovering sprayed fluorescent tracers from artificial and plant surfaces through washing techniques and determining deposition through fluorometry and colorimetry (Lake, 1988; Salyani and Whitney, 1988; 1990). These methods lack the ability to quantify the quality of coverage, such as uniformity of spray coverage on the target surface (Juste *et al*., 1990). Spray deposition measurement, specifically in terms of quantity and quality, was greatly improved through the development of deposition assessment protocols that combines fluorometry, digital photomicrographic imaging and digital image analysis (Salyani and Hoffmann, 1996; Brink *et al*., 2004; 2006; Fourie *et al*., 2009; van Zyl *et al*., 2010a; 2010b).

Alternaria Brown spot of mandarins

Alternaria brown spot (ABS) is an economically important disease of leaves, fruit and twigs of susceptible mandarin cultivars, tangerine (*Citrus reticulata* Blanco) and tangerine × grapefruit (*C. reticulata* × *C. paradisi* Macfad.) hybrids in many citrus growing regions of the world (Kiely, 1964; Whiteside, 1976; Solel, 1991; Schutte *et al*., 1992; Timmer *et al*., 1998, 2000, 2003; Vincent *et al*., 2000; Akimitsu *et al*., 2003; Elena 2006; Reis *et al*., 2006). The causal agent of ABS is the necrotrophic fungus, tangerine pathotype of *A. alternata*, *Alternaria alternata* (Fr: Fr) Keissl. pv. *citri,* that produces the host selective/specific ACT toxin (Solel 1991; Simmons, 1999a and b; Timmer *et al*., 2003; Lin et *al*., 2009).

The disease was first reported in 1903 in the coastal-citrus producing regions of Australia on Emperor mandarin fruit and leaves (Pegg, 1966; Keily, 1964; Simmons, 1999a, b). In 1973, the disease was recorded on Dancy tangerines in Florida, United States of America (Whiteside 1976; Simmons, 1999a, b; Timmer *et al*., 2000). From 1975 onward, the disease became widespread through the citrus-producing regions of North America and also the rest of the world. In Israel, ABS was reported on Minneola Tangelo in 1989 (Solel, 1991). Later it was also found in other Mediterranean citrus-producing countries, like Turkey (1995), Spain (1998) and Italy (2000) (Canihos *et al*., 1999; Vicent *et al*., 2000) and Greece (2003) (Elena, 2006).

In South Africa, severe fruit drop and loss was ascribed to ABS in the 1991/1992 growing season, reporting the presence of the disease in the country (Swart *et al*., 1998). In February 1996, the disease was first reported on Star Ruby grapefruit in an orchard near Nelspruit (Schutte, 1996). In 2003, the disease was reported in South America (Timmer *et al*., 2000; Peres *et al*., 2003).

Causal Organism

There are four distinct diseases caused by *Alternaria* species on citrus – Alternaria brown spot (ABS) of tangerines and their hybrids, Alternaria leaf spot of Rough Lemon, Alternaria black rot of fruit, and Mancha foliar on Mexican lime (Timmer *et al*., 2003).

ABS is caused by the "tangerine" pathotype, *Alternaria alternata* (Fr: Fr) Keissler pv. *citri*. The causal organism of ABS, together with several small-spored *Alternaria* spp. associated with citrus, have been identified and described as *Alternaria citri* Ellis and Pierce (Kiely, 1964; Pegg, 1966; Whiteside, 1976; Kohmoto *et al.,* 1979). Kiely (1964) and Pegg (1966) concluded that strains causing ABS and citrus black rot were morphologically identical (Peever *et al*., 2005). Description and comparison of the pathogen was done on morphology of detached conidia. Simmons (Simmons, 1999a, b), however, deemed this method unfit for taxonomic differentiation of small-spored *Alternaria*. Due to the different pathogenic symptoms and toxin production of the isolate, the causal agent of ABS was later re-named *A. alternata* (Fr: Fr) Keissler based on the conidial morphology and conidial measurements published by Simmons in 1967 (Simmons, 1999a, b). Solel (1991) suggested that the nomenclature proposed by Nishimura and Kohmoto (1983) should be adopted and the causal agent was hence forth named *A. alternata* citrus pathotype (Solel 1991).

The classification of the causal agent of ABS is therefore still vague. To clarify classification, morphological species were described through morphological species concepts (conidium catenulation and conidium morphology) on ABS-causing isolates collected from Minneola tangelo and rough lemon from different citrus-growing regions around the world in Simmons (1999a, b). This method failed to differentiate the ABS-causing isolates from other small-spored *Alternaria* spp. that is pathogenic on citrus (Simmons, 1999a, b; Peever *et al*., 2002, 2004). ABS-causing isolates can only be differentiated from other small-spored *Alternaria* by using pathogenicity tests, toxin assays or genetic markers (Peever *et al*., 1999; Akimitsu *et al*., 2003).

Symptoms

Alternaria brown spot (ABS) infect the leaves, twigs and fruit of susceptible tangerine and tangerine × grapefruit hybrids and cultivars (Kiely, 1964; Whiteside, 1976; Solel, 1991; Schutte *et al*., 1992; Timmer *et al*., 1998, 2000, 2003; Akimitsu *et al*., 2003; Vincent *et al*., 2000; Elena 2006; Reis *et al*., 2006). Most symptoms have been described on Mineola tangelo.

Young leaves are most susceptible from leaf formation until full leaf expansion and hardening (Pegg, 1966; Whiteside, 1976; Solel and Kimchi, 1998). Mature leaves are less prone to infection with susceptibility decreasing with aging of leaves (Pegg, 1966; Whiteside, 1976; Solel and Kimchi, 1998). On young flush, symptoms can appear 24 to 36 hours after infection in the form of minute brown to black necrotic spots on the leaf surface. These spots

expand in diameter and form large lesions that overlap each other, which later can cause leaf drop. Lesions are formed through chlorosis and necrosis along the leaf veins. This is because of the host-specific ACT-toxin produced by the causal agent. The toxin is translocated acropetally through the leaf, causing symptoms as the toxin spread (Whiteside, 1976; Kohmoto *et al*., 1993; Timmer *et al*., 2003). On mature leaves, brown to black necrotic spots appear surrounded by green to yellow halos. Necrotic leaf areas can also fall out of the infected leaf, causing a "shot-hole" appearance (Timmer *et al*., 2003).

On young shoots, brown lesions are formed that range from 1 to 10 mm in diameter. These lesions will expand, forming elongated cankers, causing die-back of the young shoots, after affected leaves on the shoots have abscised (Whiteside, 1976; Kohmoto *et al*., 1993; Swart *et al*., 1998; Timmer *et al*., 2003).

Fruit are susceptible from petal fall right through to full maturity. However, as fruit size increase, susceptibility decreases, depending on the severity of infection (Vicent *et al*., 2004). The lesions on fruit range from slightly depressed, minute necrotic spots to large crater-like lesions that later on form sunken pockmarks that becomes corky and can become dislodged. Fruit smaller than 2 cm often abscise and drop after a few days. Older infected fruit usually stay on the tree for weeks but abscise before maturity and also drop (Whiteside, 1976; Swart *et al*., 1998; Akimitsu *et al*., 2003; Bhatia *et al*., 2003; Timmer *et al*., 2003; Vicent *et al*., 2004). Secondary fruit rot can develop in lesions (Whiteside, 1976). The severity of infection by ABS can affect tree growth, particularly because of leaf drop. Because of fruit drop and undesirable blemishes on fruit, crop loss can be high and the marketability of fruit is thus reduced (Timmer *et al*., 2000).

Disease cycle and epidemiology

The *Alternaria* spp. is a very robust group of pathogens in terms of environmental flexibility. Their thick walled, multicellular, pigmented conidia can tolerate extremes in terms of weather and survive harsh and unfavourable environmental conditions for extended periods of time. *Alternaria alternata* can thrive at high temperatures under high rainfall conditions and also under arid conditions where there is little or no rain for certain parts of the year (Timmer *et al*., 1998).

The disease cycle is relatively simple because no known teleomorph exists for *A. alternata* (Timmer *et al*., 1998; Simmons, 1999a and b). Conidia are produced on infected leaves, twigs and fruit on the tree and also on fallen leaves and fruit. The primary source of inoculum is conidia produced on advanced lesions on young "flush" and mature (early infections) leaves (Canihos *et al*., 1999; Timmer *et al*., 1998, 2000, 2003; Vicent *et al*., 2009). Infected twigs on the tree are also an important source of inoculum. Fallen leaves, fruit and twigs serve as overwintering sites for inoculum with the latter being the most important since the leaves

disintegrate on the orchard floor and most fruit inoculum sources are removed during harvest (Whiteside, 1976; Reis *et al*., 2006).

Young lesions on infected leaves, fruit and twigs rarely produce conidia. Conidium production is greatest at high temperatures, high relative humidity and on lightly moistened leaves. On wet to very wet leaves, conidium production is lower. At moderate to low relative humidity, on dry leaves, no to little conidia are produced. Release of conidia from sporulating lesions is triggered by rainfall in humid areas, a sudden drop in relative humidity or the drying of leaves in semi-arid to -arid growing regions. (Canihos *et al*., 1999; Timmer *et al*., 1998, 2000, 2003; Vicent *et al*., 2009).

The conidia are dispersed by means of wind and rain to susceptible material. For infection to take place, the average day temperature must be between 22 to 27°C, with 27°C being the optimum. Temperatures above 32°C are too high and little to no infection will occur. Wet susceptible material from rainfall, irrigation or dew are needed for 4 to 8 h at suitable temperatures for infection to occur. At wetness periods of 10 to 12 h substantial infection can occur. Reis *et al*. (2006) showed that wet periods of up to 36 h is optimal for infection, especially in semi-arid to -arid growing regions where night temperatures are low. The lower the temperature, the longer wetness period is needed for infection to take place (Canihos *et al*., 1999; Timmer *et al*., 1998, 2000, 2003; Vicent *et al*., 2009; Reis *et al*., 2006).

When conditions are favourable for infection the spores germinate, forming an appressoria for direct penetration of fruit and leaf surfaces. Penetration is also possible through stomatal openings, especially on lower leaf surfaces where stomata are abundant. As the spores germinate and penetrates, the host selective toxin, ACT-toxin (named after *A. citri* tangerine pathotype), is released to help with the infection process (Canihos *et al*., 1999; Timmer *et al*., 2003). As motioned, the ACT-toxin causes venial necrosis.

Kohmoto *et al*. (1993) stated that the mode of action of the ACT toxin is still uncertain, but a rapid loss of electrolytes from leaf tissues and ultra-structural changes of cells by the toxin suggests that the primary action site of the toxin is likely the plasma membrane (Kohmoto *et al*., 1993; Timmer *et al*., 2003). Lin *et al*. (2009) identified two homolog genes, AaAP1 and AaFUS3, which transcribe the secretion of ACT-toxin through the mediated MAPK (Mitogen Activated Protein Kinase) signalling cascade in response to environmental stimuli. These two genes also regulate other important pathogenicity factors, such as proliferation, conidial formation, fungal penetration, appressorium formation, melanin and other hydrolytic enzyme production. It also mediates resistance to copper fungicides and other diverse chemicals and salt tolerance (Lin *et al*., 2009).

Host Specificity

Susceptibility of citrus types, cultivars and their hybrids is subject to it susceptibility to the host specific ACT toxin that *A. alternata* pv. *citri*produce (Whiteside, 1976; Kohmoto *et al*., 1991, 1993; Peever *et al*., 2003). ACT toxin production is specific to the tangerine and hybrids of tangerine (Kohmoto *et al*., 1979). Gardner *et al*. (1986), Solel and Kimchi (1997), Peever *et al.* (2000), Vicent *et al*. (2004) and Reis *et al*. (2007) have evaluated the susceptibility of citrus species to *A. alternata*. They found that most *Citrus reticulata* (tangerine) cultivars and hybrids are susceptible.

Integrated Disease Management

The presence of Alternaria brown spot (ABS) in citrus producing regions around the world threatens cultivation of cultivars susceptible to the pathogen. If the pathogen is not controlled, losses in yield (between 30 to a 100%) can be the outcome because of fruit blemishes, fruit drop and the reduced production capability of affected citrus trees.

Because of the sporadic nature of the disease when environmental conditions are favourable, the effective overwintering of large amounts of potential inoculum on dead twigs and leaves on the orchard floor and the short incubation period, the disease can easily become epidemic. For example, during the 1990/1991 growing season severe fruit loss, rind blemish, defoliation of trees and die-back of twigs were experienced because of a severe Alternaria brown spot infestation in Tzaneen, Northern Province, South Africa that caused massive income losses. This outbreak of the disease was because of ineffective disease control (Swart *et al*., 1999). Regular fungicide applications together with cultural practices are needed to produce quality healthy fruit for the export market (Vicent *et al*., 2004; Timmer *et al*., 2003).

Chemical control

The fast growing fruit and foliage of susceptible cultivars have to be protected with the use of a multiple foliar chemical spray program from petal fall (September/October) until after end of summer (March/April) to control the disease and assure acceptable yields through unblemished fruit but also to protect the foliage and shoots that can become infected and increase the build-up of inoculum of the next growing season (Schutte and Beeton, 1994; Swart *et al*., 1998; Timmer *et al*., 2003; Vicent *et al*., 2004). The chemical control strategy in South Africa and in other citrus-producing countries (depending on availability of registered products) is to assure adequate fungicide coverage of the fast-growing ABS susceptible fruit and foliage by applying the correct fungicide combination at spray intervals scheduled by taking climatic conditions, tree phenology and withholding period of selected registered fungicides into consideration (Swart *et al*., 1998). Chemical products used to control ABS include the dithiocarbonates, iprodione, copper fungicides and the strobilurins (Timmer *et al*.,

2003). These products deliver effective control but run the risk of developing fungicide resistance. It is therefore important to alternate or mix the fungicides, especially the strobilurins and iprodione with protectant fungicides (Peres *et al*., 2006). Solel *et al*. (1996) reported iprodione resistance of an *A. alternata* population in an orchard in Israel subjected to continual excessive (4 years) iprodione alone sprays.

The effectiveness of various contact and systemic fungicides has been evaluated over the years. Schutte and Beeton (1994) evaluated six fungicides in Citrusdal (Western Cape, South Africa) for the control of ABS. Triazoles (difenoconazole, bromuconazole, tebuconazole and flusilazole) used in combination with mancozeb, following copper oxychloride sprays alone, improved ABS control compared with mancozeb and copper alone sprays. The use of contact and systemic fungicides together can reduce the risk of resistance build-up against the systemic fungicides.

Solel *et al*. (1996) evaluated iprodione resistance of an *A. alternata* population in an orchard in Israel subjected to continual excessive (4 years) iprodione alone sprays. Thus, the population were subjected to iprodione resistance selection due to a poor spray program, indicating the importance of a safe spray strategy in which systemic fungicides should be alternated with other 'mode of action' fungicides and sprayed together with contact fungicides to reduce resistance build-up against systemic fungicides.

Timing of application of fungicide sprays is important (Swart, 1998). Contact fungicides such as copper oxychloride, copper hydroxide and cuprous oxide are sprayed together with a registered systemic fungicide to reduce the risk of build-up of resistance against systemic fungicides used. With most sprays, if not all, a surfactant is added to improve fungicide deposition on the target surface, penetration into the dense citrus canopy, uniformity of spray application throughout the canopy, and biological efficacy of fungicides sprayed for ABS control. The use of surfactants together with fungicide sprays was evaluated in this study.

The Alter-Rater model was developed to time fungicide applications for disease control. The use of this system potentially reduces the number of fungicide applications. The model uses a point system in which each day is assigned a value for favourability of disease. Contributing factors such as rain, duration of leaf wetness and temperature taken into account by the point system. The points are accumulated daily and a fungicide application is applied as soon as a predefined value is reached (Timmer *et al*., 2000).

Cultural practices

Cultural practices can help to reduce disease severity. However, cultural practices are not sufficiently adequate to reduce disease severity by itself. It does, however, enhance the efficiency of fungicide control programmes (Timmer *et al*., 2003). Cultural practices should aim at reducing leaf and canopy wetness, which should reduce the amount of disease. Thus,

practices that increase leaf and canopy wetness like overhead irrigation should be avoided. Planting orchards in a north-south direction on higher locations where air draining is better and with wider spacing for better airflow, will minimise disease pressure because the tree canopy will dry more readily (Whiteside, 1976; Timmer *et al*., 2003). Over-irrigation and too much nitrogen fertilisation must be avoided to reduce the production of susceptible young foliage (Akimitsu *et al*., 2003).

Choice of rootstock is important. Choosing less vigorous rootstocks for plantings will lower vegetative growth. This reduces the amount of material susceptible to disease. It also lowers the chance for build-up of inoculum. Light hedging and cutting of windows in the canopy should be done frequently for improved aeration and spray penetration of the inner canopy. It also reduces young shoot growth that in turn reduces canopy density but can conversely stimulate flush growth (Timmer and Peever, 1997). Pruning debris has to be removed from the orchard. If possible, fallen leaves must also be removed from the orchard floor. Dead wood from trees must also be cut out. These actions will reduce overwintering material for the pathogen. This will reduce the pathogen population and possible infection levels for the next growing season (Timmer and Peever, 1997).

CONCLUSION

Adjuvants are used regularly in fungicide spray application in citrus. However, as indicated in literature, the effect it has on fungicide and pesticide deposition varies depending on crop type (Steurbaut, 1993; van Zyl *et al*., 2010a; 2010b; Gaskin *et al*., 2004, 2006, 2008; van Zyl *et al*., 2014; Decaro *et al*., 2016). Thus, the assumption that it improves deposition on all types of target surfaces cannot be made since target surfaces, be it leaves or fruit, vary in composition and structure depending on crop type (De Ruiter *et al*., 1990). It is therefore important to study the influence adjuvants have on fungicide deposition in citrus since it is unknown.

To study fungicide deposition effectively, and the influence adjuvants have on deposition parameters, methodology is needed to evaluate deposition on target surfaces in terms of quantity and quality and the uniformity between targets. Improvement of currently used spray deposition evaluation protocols such as that developed by Brink *et al* (2004; 2006), Fourie *et al*. (2009) and van Zyl *et al*. (2010a and b) and used by Schutte *et al*. (2012) and van Zyl *et al*. (2014) is required for high throughput deposition assessment on citrus leaves.

Deposition data alone will show how adjuvants influenced physical deposition parameters. However, as plant pathologists we are interested as to how deposition parameters relate to disease control. Development of deposition benchmarks indicative of biological efficacy of fungicides will enable this. *Alternaria alternata* used as a model pathogen together with its sensitivity to copper oxychloride will be able to simulate this effectively.

This study will therefore have the following objectives: to describe a novel deposition assessment protocol for the assessment of spray deposition quantity, quality and uniformity, specifically for use in citrus spray application research; to determine deposition benchmarks indicative of biologically effective deposition quantities; to use the deposition assessment protocol and benchmarks to evaluate the influence of adjuvants on deposition in citrus orchards; and to determine what influence adjuvants have on disease control in a laboratory study.

REFERENCES

Act 36 of 1946. Fertilizers, farm feeds, agricultural remedies and stock remedies act 36 of 1947.

Akimitsu, K., Peever, T.L., Timmer, L.W., 2003. Molecular, ecological and evolutionary approaches to understanding *Alternaria* diseases of citrus. Molecular Plant Pathology 4: 435-446.

ASTM. American Society for Testing and Materials E1519-16. Annual book of ASTM standards. Standard terminology relating to tank mix adjuvants. ASTM International, West Conshohocken, PA, US pages 1 to 3.

Baker, E.A., Procopiou, J., Hunt, G.M. 1975. The cuticles of *Citrus* species. Composition of leaf and fruit waxes. Journal of the Science of Food and Agriculture 26: 1093-1101.

Bargel, H., Cerman, Z., Koch., Koch, K., Netnhuts, C., 2006. Structure-function relationships of the plant cuticle and cuticular waxes – a smart material? Functional Plant Biology 33: 893-910.

Bhatia, A., Roberts, P.D., Timmer, L.W. 2003. Evaluation of the Alter-Rater model for timing fungicide applications for control of Alternaria brown spot of citrus. Plant Disease 87: 1089-1093.

Brink, J.C., Calitz, F.J., Fourie, P.H., Holz, G., 2004. Development of a protocol to quantify spray deposits on grape bunches. 7th International Symposium on Adjuvants for Agrochemicals ISAA 2004, ISAA 2004 Foundation, South Africa, 230-236.

Brink, J.C., Fourie, P.H., Holz, G., 2006. Effect of fungicide spray cover on *Botrytis cinerea* infection in grape bunches. South African Journal of Enology and Viticulture 27: 51-56.

Butler Ellis, M.C., Tuck, C.R., Miller, P.H.C. 1997. The effect of some adjuvants on sprays produced by agricultural flat fan nozzles. Crop Protection 16: 41-50.

Butler Ellis, M.C., Tuck, C.R. 1999. How adjuvants influence spray formulation with different hydraulic nozzles. Crop Protection 18:101-109.

Butler Ellis, M.C., Bradley, A. 2002. The influence of formulation on spray drift. Aspects of applied biology, International Advances in Pesticide Application 66: 251-258.

Byass, J.B. 1969. Equipment and methods for orchard spray application research. Journal of Agricultural Engineering research 14: 78-88.

Byers, R.E., Barden, J.A., Donohue, S.J., Horsburgh, R.L., Lyons jr., C.G., Yoder, K.S., 1984. Effects of apple tree size and canopy density on spray chemical deposit. HortScience 19: 93-94.

Byers, R.E. 1987. Tree-Row-Volume spraying calculator for apples. American Society for Horticultural Science 22: 506-507.

Canihos, Y., Peever, T.L., Timmer, L.W., 1999. Temperature, leaf wetness, and isolate effects on infection of Minneola tangelo leaves by *Alternaria* sp. Plant Disease. 83: 429-433.

Cooke, B.K. and Hislop, E.C. 1993. Spray tracing techniques. Pages 85-100 in: Application technology for crop protection. Matthews, G.A., Hislop, E.C. Wallingford: CAB.

Cromwell, R.P. 1975. Citrus growers guide to air spraying. University of Florida, IFAS Extension Circular 351.

Cross, J.V., Walklate, P.J., Murray, R.A., Richardson, G.M. 2001a. Sprayer deposits and losses in different sized apple trees from an axial fan orchard sprayer: 1. Effects of spray liquid flow rate. Crop Protection 20: 13-30.

Cross, J.V., Walklate, P.J., Murray, R.A., Richardson, G.M. 2001b. Sprayer deposits and losses in different sized apple trees from an axial fan orchard sprayer: 2. Effects of spray quality. Crop Protection 20: 333-343.

Cross, J.V., Walklate, P.J., Murray, R.A., Richardson, G.M. 2001c. Sprayer deposits and losses in different sized apple trees from an axial fan orchard sprayer: 3. Effects of air volumetric flow rate. Crop Protection 20: 333-343.

Cunha, J.P., Chueca, P., Garcerá, C., Moltó, E. 2012. Risk assessment of pesticide spray drift from citrus applications with air-blast sprayers in Spain. Crop Protection 42: 116-123.

Cunningham, G.P., Harden, J., 1998a. Air-tower sprayers increase spray application efficiency in mature citrus trees. Australian Journal of Experimental Agriculture. 38: 871-877.

Cunningham, G.P., Harden, J., 1998b. Reducing spray volumes applied to mature citrus trees. Crop Protection 17: 289-292.

Cunningham, G.P., Harden, J., 1999. Sprayers to reduce spray volumes in mature citrus trees. Crop Protection 18: 275-281.

Decaro, R. A., Decaro, S.T.D Jr., da Costa Ferreira, M. 2016. Deposit of pesticides without and with adjuvants on citrus seedlings following different intervals of artificial rain. Ciência Rural, Santa Maria 46: 13-19.

de Jong, F.M.W., de Snoo, G.R., van der Zande, Jan C. 2008. Estimated nationwide effects of pesticide drift on terrestrial habitats in the Netherlands. Journal of Environmental Management 86: 721-730.

de Ruiter, H., Uffing, A.J.M., Meinen, E., Prins, A., 1990. Influence of surfactants and plant species on leaf retention of spray solutions. Weed Science 38: 567-572.

Derksen, R.C., Zhu, H., Fox, R.D., Brazee, R.D., Krause, C.R. 2007. Coverage and drift produced by air induction and conventional hydraulic nozzles used for orchard applications. Transactions of the American Society of Agricultural and Biological Engineers 50: 1493-1501.

Dorr, G.J., Wang, S., Mayo, L.C., McCue, S.W., Forster, W.A., Hanan, J., He, X. 2015. Impaction of spray droplets on leaves: influence of formulation and leaf character on shatter, bounce and adhesion. Experiments in Fluids 56:1-17.

Edmonds, J. 2018. Key Industry Statistics for Citrus Growers. 2017 Export season. Citrus Growers Association. Hillcrest, Kwazulu-Natal, South Africa. www.cga.co.za.

Elad, Y., Aynish, N., Ziv, O., Kata, J., 1990. Control of grey mold (*Botrytis cinerea*) with film-forming polymers. Plant Pathology 39: 249-254.

Elena, K., 2006. Alternaria brown spot of Minneola in Greece; evaluation of citrus species susceptibility. European Journal of Plant Pathology 115: 259-262.

Farooq, M., Salyani, M., 2002. Spray penetration into the citrus tree canopy from two air-carrier sprayers. Transactions of the American Society of Agricultural Engineers 45: 1287-1293.

Furness, G.O., Drew, H.J., Magarey, P.A., Miller, P.H., 1998. Fruit tree and vine sprayer calibration based on canopy size and length of row: Unit canopy row method. Crop Protection 17: 639-644.

Furness, G.O., Manktelow, D.W.L., Thompson, A.J., 2006a. A Visual droplet number rating chart and fluorescent pigment sprays to estimate chemical deposition and spray coverage on plant foliage. Aspects of Applied Biology: 77: 171-178.

Furness, G.O., Manktelow, D.W.L., Thompson, A.J., 2006b. Multi-fan spray towers to improve dose efficiency and spray coverage uniformity in citrus trees. Aspects of Applied Biology 77: 481-488.

Fourie, P.H., du Preez., M., Brink, J.C., Schutte, G.C. 2009. The effect of run-off on spray deposition and control of Alternaria brown spot of mandarins. Australasian Plant Pathology 38: 173-182.

Gale, J., Hagan, R.M. 1996. Plant antitranspirants. Annual Review of Plant Physiology 17: 269-282.

Gaskin, R.E., Elliott, G.S., Manktelow, D.W.L., Skinner, S.J., 2004. Use of a superspreader adjuvant to reduce spray application volumes on avocados. New Zealand Plant Protection 57: 266-270.

Gaskin, R. E., Elliott, G.S., Manktelow, D.W.L., Steele, K.D., 2006. Adjuvant and application technologies to minimise off-target drift from kiwifruit sprays. New Zealand Plant Protection 59: 217-222.

Gaskin, R.E., Manktelow, D.W.L., Pak, H., 2008. Improving spray distribution and coverage on avocado trees in low volume, concentrate sprays. New Zealand Plant Protection 61: 147-152.

Gaskin, R.E., Steele, K.D., 2009. A comparison of sticker adjuvants for their effects on retention and rianfastning of fungicide sprays. New Zealand Plant Protection 62: 339-342.

Garcerá, C., Moltó, E, Chueca, P. 2011. Effect of spray volume of two organophosphate pesticides on coverage and mortality of California red scale Aonidiella aurantia (Maskell). Crop Protection 30: 693-697.

Garcerá, C., Moltó, E., Chueca, P. 2014. Factors influencing the efficacy of two organophosphate insecticides in controlling California red scale, *Aonidiella aurantia* (Maskell). A basis for reducing spray application volume in Mediterranean conditions. Pesticide Management Science 70: 28-38.

Garcerá, C., Fonte, A., Moltó, E., Chueca, P. 2017. Sustainable use of pesticide applications in citrus: A support tool for volume rate adjustment. International Journal of Environmental Research and Public Health 14: 1-14.

Gardner, J.M., Kono, Y., Chandler, J.L. 1986. Bioassay and host-selectivity of *Alternaria citri* toxins affecting rough lemon and mandarins. Physiological and Molecular Plant Pathology 29: 293-304.

Gent, D.H., Schwartz, H.F., Nissen, S.J., 2003. Effect of commercial adjuvants on vegetable crop fungicide coverage, absorbtion and efficacy. Plant Disease 87: 591-597.

Georgala, M.B. 1975. Possible resistance of red scale *Aonidiella aurantii* Mask to corrective spray treatments. Citrus and Subtropical Fruit Journal 505: 5-17.

Gregorio, E., Torrent, X., de Marti, P.S., Solanelles, F., Sanz, R., Rocadenbosch, F., Masip., J, Ribes-Dasi, M., Rosell-Polo, J.R. 2016. Measurement of spray drift with spesifically designed Lidar system. Sensors 16: (499) 1-15.

Green, J.M., Beestman, G.B. 2007. Recently patented and commercialized formulation and adjuvant technology. Crop Protection 26: 320-327.

Grout, T.G., 1997. Spray volumes and coverage requirements for citrus in South Africa. Citrus J. 31: 19-20.

Grout, T.G., 2003. Use of plant protection products. Section 2.7. In: Grout, T.G. (Ed.), Citrus Production Guidelines. Integrated Pest Management, vol. III. Citrus Research International, Nelspruit, South Africa.

Hazen, J.L. 2000. Adjuvants-Terminology, Classification and Chemistry. Weed Technology 14: 773-784.

Hock, W.K. 1998. Horticultural spray adjuvants. Chapter 10 in: Agrochemical Fact Sheets. Penn State College of Agricultural Sciences Research; Pennsylvania State University, Pennsylvania, United States of America

Hoffmann, W.C., Salyani, M. 1996. Spray deposition on citrus canopies under different meteorological conditions. Transactions of the American Society of Agricultural Engineers 39: 17-22.

Holloway, P.J., Butler Ellis, M.C., Webb, D.A., Western, N.M., Tuck, C.R., Hayes, A.L. and Miller, P.C.H. 2000. Effects of some agricultural tank-mix adjuvants on the deposition efficiency of aqueous sprays on foliage. Crop Protection 19: 27-37.

Holownicki, R., Doruchowski G., Jaeken, P., Świechowski, W., 2002. Methods of evaluation of spray deposit and coverage on artificial targets. Electronic Journal of Polish Agricultural Universities (EJPAU) 5(1), #03. Online publication.

Jejčič, V., Godeša, T., Hočevar, M., Širok, B., Malneršič, A., Štancar, A., Lešnik, M., Stajnko, D., 2011. Design and testing of an ultrasound system for targeted spraying in orchards. Strojniški vestnik – Journal of Mechanical Engineering 57: 587-598.

Jiang, C., Derksen, C.R., 1995. Morphological image processing for spray deposit analysis. Transactions of the American Society of Agricultural Engineers 38: 1581-1591.

Juste, F., Sánchez, S., Ibáñez, R., Val, L., García, C., 1990. Measurement of spray deposition and efficiency of pesticide application in citrus orchards. Journal of Agricultural Engineering Research 46: 187-196.

Kiely, T.B., 1964. Brown spot of Emperor mandarin. The agricultural gazette of New South Wales 75: 854-856.

Kohmoto, K., Scheffer, R.P., Whiteside, J.O., 1979. Host-selective toxins from *Alternaria citri*. Phytopathology 69: 667-671.

Kohmoto, K., Itoh, Y., Shimomura, Y., Kondoh, H., Otani, M., Kodama, S., Nishimura, S., Nakatsuka, S. 1993. Isolation and biological activities of two host-specific toxins from the tangerine pathotype of *Alternaria alternata*. Phytopathology 83: 495-502.

Kotzé, J.M., 2000. Black spot. Pages 23-25 in: Compendium of Citrus Diseases (second Edition). Timmer, L.W., Garnsey, S.M. and Graham, J.H. APS Press, The American Phytopathological Society, St. Paul Minnesota, USA.

Kotzé, C., Moyo, P., Fourie, P.H. 2018. Updated citrus black spot spray programmes. CRI Cutting Edge No 237.

Kirkwood, R.C. 1993. Use and mode of action of adjuvants for herbicides: A review of some current work. Pesticide Science 38: 93-102.

Lake, J.R., 1988. The deposition of electrostatically charged sprays on parts of targets shaded from the spray. Journal of Agricultural Engineering Research 39: 9-18.

Landers, A.J. 2010. Effective orchard spraying. A practical guide for growers. Effective Spraying. Ithaca, New York, USA.

Larbi, P.A., Salyani, M. 2012. CitrusSprayEx: An expert system for planning citrus spray applications. Computers and Electronics in Agriculture 87: 85-93.

Lin, C-H., Ling Yang, S., Chung, K., 2009. The YAP1 homolog-mediated oxidative stress tolerance is crucial for pathogenicity of the necrotrofic fungus *Alternaria alternata* in citrus. Molecular Plant-Microbe Interactions 22: 942-952.

Mayo, L.C., McCue, S.W., Moroney, T.J., Forster, W.A., Kempthorne, D.M., Belward, J.A., Turner, I.W. 2015. Simulating droplet motion on virtual leaf surfaces. Royal Society of Open Science 2: 140528.

McMullan, P.M. 2000. Utility adjuvants. Weed Technology14: 792-797.

Meli, S., Renda, A., Nicelli, M., Capri, E. 2003. Studies on pesticide drift in Mediterranean citrus area. Agronomie, EDP Sciences 23: 667-672.

Nishimura, S., Kohmoto, K. 1983. Host-specific toxins and chemical structures from *Alternaria* species. Annual Review of Phytopathology 21: 87-116.

Nuyttens, D., De Shampheliere, M., Steurbaut, W., Baetens, K., Verboven, P., Nicolaï, B., Ramon, H., Sonck, B. 2006. Experimental study of factors influencing the risk of drift from field sprayers, Part1: Meteorological conditions. Aspects of Applied biology 77: 1-8.

Nuyttens, D., Beatens, K., De Schampheleire, M., Sonck, B. 2007. Effect of nozzle type, size and pressure on spray droplet characteristics. Biosystems Engineering 97: 333-345.

Peever, T.L., Canihos, Y., Olsen, L., Ibañez, A., Liu, Y.C., Timmer, L.W. 1999. Population genetic structure and host specificity of *Alternaria* spp. Causing brown spot of minneola tangelo and rough lemon in Florida. Phytopathology 89: 851-860.

Peever, T.L., Olsen, L., Ibañez, A., Timmer, L.W. 2000. Genetic differentiation and host specificity among populations of *Alternaria* spp. causing brown spot of grapefruit and tangerine × grapefruit hybrids in Florida. Phytopathology 90: 407-414.

Peever, T.L., Ibañez, A., Akimitsu, K., Timmer, L.W. 2002. Worldwide Phylogeography of the citrus brown spot pathogen, *Alternaria alternata*. Phytopathology 92: 794-802.

Peever, T.L., Su, G., Carpenter-Boggs, L., Timmer, L.W. 2004. Molecular systematics of citrus-associated *Alternaria* species. Mycologia 95: 119-134.

Peever, T.L., Carpenter-Boggs, L., Timmer, L.W., Carris, L.M., Bhatia, A. 2005. Citrus black rot is caused by phylogenetically distinct lineages of *Alternaria alternata*. Phytopathology 95: 512-518.

Pegg, K.G. 1966. Studies of a strain of *Alternaria citri* Pierce, the causal organism of brown spot of Emperor mandarin. Queensland Journal of Agriculture and Animal Sciences 23: 15-28.

Percival, G.C., Boyle, S. 2009. Evaluation of film forming polymers to control apple scab (*Venturia inaequalis* (Cooke) G. Wint.) under laboratory and field conditions. Crop Protection 28: 30-35.

Penner, D. 2000. Activator adjuvants. Weed Technology 14: 785-791.

Reis, R.F., de Goes, A., Mondal, S.N., Shilts, T., Brentu, F.C., Timmer, L.W., 2006. Effect of lesion age, humidity, and fungicide application on sporulation of *Alternaria alternata*, the cause of brown spot of tangerine. Plant Disease 90: 1051-1054.

Reis, R.F., de Almeida, T.F., Stuchi, E.S., de Goes, A. 2007. Susceptibility of citrus species to *Alternaria alternata*, the causal agent of Alternaria brown spot. Scientia Horticultarae 113: 336-342.

Rosell, J.R., Llorens, J., Sanz, R., Arnó, J., Ribes-Dasi, M., Masip, J., Escolà, A., Camp, F., Solanelles, F., Gràcia, F., Gil, E., Val, L., Planas, S., Palacin, J. 2009. Obtaining the three-dimentional structure of tree orchards from remote 2D terrestrial LIDAR scanning. Agricultural and Forest Meteorology 149: 1505-1515.

Rossouw, C.J., Fourie, P.H., van Zyl., J.G., Hoffman, J.E., McLeod, A. 2018. Rainfastness of mancozeb on apple seedlings determined through deposition quantification of mancozeb residue and fluorescent pigment. Crop Protection 106: 93-102.

Salyani, M., 1994. Spray technology research for orchard applications. Acta Horticulturae 372: 67-74.

Salyani, M. 2003. Calibration of airblast sprayers.In: Circular 1435, Series of the Agricultural and Biological Engineering department, Florida Cooperative Extension Service. Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu.

Salyani, M., 2005. 2005 Florida citrus pest management guide: Pesticide Application Technology. AE-259, series of the Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu.

Salyani, M., 2006. 2006 Florida citrus pest management guide: Pesticide Application Technology. ABE-356, series of the Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu

Salyani, M. 2015. 2015 Florida Citrus Pest Management Guide: Pesticide Application Technology. In ABE-356, Series of the Agricultural and Biological Engineering department, Florida Cooperative Extension Service. Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu.

Salyani, M., Whitney, J.D., 1988. Evaluation of methodologies for field studies of spray deposition. Transactions of the American Society of Agricultural Engineers 31: 1680-1684.

Salyani, M., Whitney, J.D., 1990. Ground speed effect on spray deposition inside citrus trees. Transactions of the American Society of Agricultural Engineers 33: 361-366.

Salyani, M., McCoy, C.W., 1989. Deposition of different spray volumes on citrus trees. Proceedings of the Florida State Horticultural Society 109: 46-50.

Salyani, M., Farooq, M., 2005. Drift potential of citrus air-carrier sprayers. Proceedings of the Florida State Horticultural Society 117: 130-135.

Salyani, M., Farooq, M., Sweeb, R.D. 2007. Spray deposition and mass balance in citrus orchard applications. Transactions of the American Society of Agricultural and Biological Engineers 50: 1963-1969.

Schutte, G.C., Lesar, K.H., Pelser, P. du T., 1992. The use of tebuconazole for the control of *Alternaria alternata* on 'Minneola' Tangelos and its potential to control postharvest decay when applied as a preharvest spray. Proceedings of the International Society of Citriculture 3: 1070-1074.

Schutte, C.G., Beeton, K.V. 1994. The use of triazoles to control *Alternaria* brown spot of Minneola tangelo in the winter rainfall region of South Africa. Citrus Journal 4: 19-20.

Schutte, C.G., 1996. First report of Alternaria brown spot on Star Ruby grapefruit in South Africa. Citrus Journal 6: 24.

Schutte, G.C., Beeton, K.V., Kotzé, J.M., 1997. Rind stippling on Valencia oranges by copper fungicides used for control of citrus black spot in South Africa. Plant Disease 81: 851-854.

Schutte, G.C., Kotze, C., van Zyl, J.G., Fourie, P.H., 2012. Assessment of retention and persistence of copper fungicides on orange fruit and leaves using fluorometry and copper residue analyses. Crop Protection 42: 1-9.

Schutte, T., le Roux, H and Fourie, P.H. 2014. Citrus black spot spray programmes. Cutting Edge 189.

Simmons, E.G. 1999a. Alternaria themes and variations – Classification of citrus pathogens. Mycotaxon 70: 226-235.

Simmons, E.G. 1999b. Alternaria themes and variations – Host-specific toxin producers. Mycotaxon 70: 236-243.

Solanelles, F., Escola, A., Planas, S., Rosell, J.R., Camp, F., Gracia, F. 2006. An electronic control system for pesticide application proportional to the canopy width of tree crops. Biosystems Engineering 95: 473-481.

Solel, Z., 1991. Alternaria brown spot on Minneola tangelos in Israel. Plant Pathology 40: 145-147.

Solel, Z., Timmer, L.W., Kimchi, M. 1996. Iprodione resistance of *Alternaria alternat*a pv. *citri* from Minneola tangelo in Israel and Florida. Plant Disease 80: 291-293.

Solel, Z., Kimchi, M. 1997. Susceptibility and résistance of citrus genotypes to *Alternaria alternata* pv. *citri*. Journal of Phytopathology 145: 391-398.

Solel, Z., Kimchi, M. 1998. Histopathology of infection of Minneola tangelo by Alternaria alternata PV. Citri and the effect of host and environmental factors on lesion development. Journal of Phytopathology 146: 557-561.

Staniland, L.N. 1959. Fluorescent tracer techniques for the study of spray and dust deposits. Journal of Agricultural Engineering research 4: 110.

Steurbaut, W. 1993. Adjuvants for use with foliar fungicides. Pesticide Science 38: 85-91.

Stevens, P.J.G., Kimberley, M.O., Murphy, D.S., Policello, G.A., 1993. Adhesion of spray droplets of foliage: the role of dynamic surface tension and advantages of organosilicone surfactants. Pesticide Science 38: 237-245.

Stock, D., Holloway, P.J. 1993. Possible mechanisms for surfactant-induced foliar uptake of agrochemicals. Pesticide Science 38: 165-177.

Stock, D., Briggs, G. 2000. Physiochemical properties of adjuvants: Values and applications. Weed Technology 14: 798-806.

Stover, E., Salvatore, J. 2002. Influence of spray volume, ground speed and a sensor-actuated spray controller on melanose control and fruit marking in fresh Indian river grapefruit. Proceedings of the Florida State Horticultural Society 115: 1-5.

Stover, E., Scotto, D., Wilson, C., Salyani, M., 2002. Spray application to citrus: Overview of factors influencing spraying efficacy and off-target deposition. HS-851, series of the Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu.

Stover, E., Scotto, D., Wilson, C., Salyani, M., 2003. Pesticide spraying in the Indian River Grapefruit: II. Overview of factors influencing spray efficacy and off-target deposition. Hortechnology 13: 166-177.

Sundaram, K.M.S., Sundaram, A., 1987. Role of formulation ingredients and physical properties on droplet size spectra, deposition, and persistence of aerially sprayed amonicarb and mexacarbate in forest litter soil samples. Pages 139-151 in: Pesticide Formulations and Application Systems: Seventh Volume (G. B. Beestman and D.I.B Vander Hooven, Eds). American Society for Testing and Materials, Philadelphia, United States of America.

Sutton, T.B., Unrath, C.R. 1984. Evaluation of the tree-row-volume concept with density adjustments in relation to spray deposits in apple orchards. Plant Disease 68: 480-484.

Sutton, T.B., Unrath, C.R. 1988. Evaluation of the tree-row-volume model for full-season pesticide application on apples. Plant Disease 72: 629-632.

Swart, S.H., Wingfield, M.J., Swart, W.J., Schutte, G.C., 1998. Chemical control of Alternaria brown spot on Minneola tangelo in South Africa. Annals of Applied Biology 133: 17-30.

Timmer, L.W., 2000. Alternaria brown spot of Mandarins. Pages 19-20 in: Compendium of Citrus Diseases (second Edition). Timmer, L.W., Garnsey, S.M. and Graham, J.H. APS Press, The American Phytopathological Society, St. Paul Minnesota, USA.

Timmer, L.W., Peever, T.L. 1997. Managing Alternaria brown spot. Citrus Industry 78: 24-25.

Timmer, L.W., Solel, Z., Gottwald, T.R., Ibañez, A.M. and Zitko, S.E. 1998. Environmental factors affecting production, release, and field populations of conidia of *Alternaria alternata*, the cause of brown spot of citrus. Phytopathology 88: 1218-1223.

Timmer, L.W., Darhower, H.M., Zitko, S.E., Peever, T.L., Ibanez, A.M., Bushong, P.M., 2000. Environmental factors affecting the severity of Alternaria brown spot of citrus and their potential use in timing fungicide applications. Plant Disease 84: 638-643.

Timmer, L.W., Peever, T.L., Solel, Z. And Akimitsu, K. 2003. Alternaria diseases of citrus - Novel pathosystems. Phytopathologia Mediterranea 42: 3-16.

Tu, M., Randall, J.M. 2003. Adjuvants. Chapter 8 in: Tu, M., Hurd, C., Randall, J.M. 2003. Weed Control Methods Handbook, The Nature Conservancy. http://tncweeds.ucdavis.edu/.

Tumbo, S.D, Salyani, M., Whitney, J.D., Wheaton, T.A., Miller, W.M. 2002. Investigation of laser and ultrasonic ranging sensors for measurement of citrus canopy volume. Applied Engineering in Agriculture 18: 367-372.

Unrath, C.R. 2002. Spray volume, canopy density, and other factors involved in thinner efficacy. American Society for Horticultural Science 37: 481-483.

van Zyl, S.A., Brink, J.C., Calitz, F.J., Fourie, P.H., 2010a. Effects of adjuvants on deposition efficiency of fenhexamid sprays to Chardonnay grapevine foliage. Crop Protection 29: 843-852.

van Zyl, S.A., Brink, J.C., Calitz, F.J., Coertze, S., Fourie, P.H., 2010b. The use of adjuvants to improve spray deposition and *Botrytis cinerea* control on Chardonnay grapevine leaves. Crop Protection 29: 58-67.

Van Zyl, J.G, Fourie, P.H., Schutte, C.G., Grout, T., van Zyl, J.G., 2013. Spray application in citrus – Sprayer calibration and checklist. CRI Cutting Edge no 166: 1-6.

Van Zyl, J.G., Sieverding, E.G., Viljoen, D.J., Fourie, P.H. 2014. Evaluation of two organosilicone adjuvants at reduced foliar spray volumes in South African citrus orchards of different canopy densities. Crop Protection 64: 198-206.

Vercruysse, F., Steurbaut, W. 2002. POCER, the pesticide occupational and environmental risk indicator. Crop Protection 21: 307-315.

Vicent, A., Armengol, J., Sales, R., García – Jiménez, J., Alfaro – Lassala, F., 2000. First report of Alternaria brown spot of citrus in Spain. Plant Disease 84: 1044.

Vicent, A., Badal, J., Asensi, M.J., Sanz, J., Armengol, J., García-Jiménez, J. 2004. Laboratory evaluation of citrus cultivars susceptibility and influence of fruit size on Fortune mandarin to infection by *Alternaria alternata* pv. *citri*. European Journal of Plant Pathology 110: 245-251.

Vicent, A., Armengol, J., García-Jiménez, J., 2009. Protectant activity of reduced concentration copper sprays against Alternaria brown spot on 'Fortune' mandarin fruit in Spain. Crop Protection 28: 1-6.

Walters, D.R. 2006. Disguising the leaf surface: the use of leaf coatings for plant disease control. European Journal of Plant Pathology 114: 255-260.

Wagner, P., Fűrstner, R., Barthlott, W., Neinhuis, C., 2003. Quantitative assessment to the structural basis of water repellency in natural and technical surfaces. Journal of Experimental Botany 54: 1295-1303.

Wei, J., Salyani, M. Development of a laser scanner for measuring tree canopy characteristics: Phase 1. Prototype development. 2004. Transactions of the American Society of Agricultural Engineers 47: 2101-2107.

Wei, J., Salyani, M. Development of a laser scanner for measuring tree canopy characteristics: Phase 2. Foliage density measurement. 2005. Transactions of the American Society of Agricultural Engineers 48: 2595-1601.

Ware, G.W., Estesen, B.J., Cahill, W.P., Gerhardt, P.D., Frost, K.R., 1969. Pesticide drift. I. High-clearance vs. aerial application of sprays. Journal of Economic Entomology 62: 840-843.

Whiteside, J.O., 1976. A newly recorded *Alternaria*-induced brown spot disease on Dancy tangerines in Florida. Plant Disease Reporter 60: 326-329.

Whiteside, J.O., Tmmer, L.W., 2000. Melanose. Page 28-29 in: Compendium of Citrus Diseases (second Edition). Timmer, L.W., Garnsey, S.M. and Graham, J.H. APS Press, The American Phytopathological Society, St. Paul Minnesota, USA.

Whitney, J.D., Churchill, D.B., Knapp, J.L., Littell, R.C., Salyani, M., Whiteside, J.O., 1988. Ground speed and spray volume of airblast sprayers affect copper deposition and greasy spot control. Proceedings of the Florida State Horticultural Society 101: 13-17.

Whitney, J.D., Salyani, M., Churchill, D.B., Knapp, J.L., Whiteside, J.O., Littel, R.C., 1989. A field investigation to examine the effects of sprayer type, ground speed and volume rate on spray deposition in Florida citrus. Journal of Agricultural Engineering Research 42: 275-283.

Whitney, J.D. Miller, W.M., Wheaton, T.A., Salyani, M., Schueller, J.K. 1999. Precision farming applications in Florida citrus. Applied Engineering in Agriculture 15: 399-403.

Yates, W.E., Akesson, N.B., Cowen, R.E., 1974. Criteria for minimizing drift residues on crops downwind from aerial applications. Transactions of the American Society of Agricultural Engineers 17: 627-632.

Zabkiewicz, J.A. 2007. Spray formulation efficacy-holistic and futuristic perspectives. Crop Protection 26: 312-319.

Zamahn, Q.U, Salyani, M. 2004. Effects of foliage density and ground speed on ultrasonic measurement of citrus tree volume. Applied Engineering in Agriculture 20: 1-6.

Zwertvaeger, I.K., Veraeghe, M., Brusselman, E., Verboven, P., Lebeau, F., Massinon, M., Nicolaï, B.M. 2014. The impact and retention of spray droplets on a horizontal hydrophobic surface. Biosystems Engineering 126: 82-91.

CHAPTER 2

Spray deposition assessment and benchmarks for control of Alternaria brown spot on mandarin leaves with copper oxychloride

This chapter has been published as: van Zyl, J.G., Fourie, P.H., Schutte, C.G., 2013. Spray deposition assessment and benchmarks for control of Alternaria brown spot on mandarin leaves with copper oxychloride. Crop Protection.46: 80-87

ABSTRACT

Inadequate disease control on citrus foliage and fruit is often attributed to insufficient fungicide spray deposition on target surfaces. This study describes a novel spray deposition assessment protocol and determines deposition benchmarks indicative of the biological effectiveness for better interpretation of spray deposition results. Suitability of a yellow fluorescent pigment as tracer for copper oxychloride deposition was demonstrated through its similar particle concentration and size. Spray deposition assessment of spray targets, which were sprayed with a mixture that included the fluorescent pigment, involved photomacrography of whole leaf or fruit surfaces, followed by digital image analyses. This protocol proved to be very accurate in determining the quantity and quality of deposition. To determine deposition benchmarks, detached young 'Nova' mandarin leaves were sprayed with copper oxychloride and fluorescent pigment at different concentrations (0.1 to 2 times the recommended concentration) and spray deposition assessed. Subsequently, leaves were spray inoculated with a spore suspension of *Alternaria alternata* [causal agent of Alternaria brown spot (ABS) of mandarins], moist-incubated for *c.* 48 h and symptom expression rated. A very good linear relationship was found between fungicide concentration, leaf area covered by fluorescent pigment particles (CV%) (r = 0.879) and Cu residue analysis (r = 0.992). A von Bertalanffy growth curve best fitted the relation between ABS control and deposition quantity (FPC%) data (91% of the percentage variance accounted for) with a good correlation between observed and predicted values (r = 0.825). Benchmarks for 50% and 75% disease control were calculated as 2.07 FPC% and 4.14 FPC%, respectively. These corresponded with Cu residue levels of 59.4 and 91.0 mg kg^{-1}, respectively. These FPC benchmarks can be used to evaluate spray technology research, specifically for control of ABS and similar citrus fruit and foliar diseases.

INTRODUCTION

In South Africa and most citrus-producing regions of the world, fruit and foliar diseases cause major economic losses, often due to the poor implementation of disease control measures.

Diseases such as Alternaria brown spot (ABS) (*Alternaria alternata* (Fr: Fr) Keissl., tangerine pathotype) (Schutte, 1996; Timmer, 2000; Timmer *et al*., 2000), citrus black spot (CBS) (*Guignardia citricarpa* Kiely) (Schutte *et al*., 1997; Kotzé, 2000), and melanose (*Phomopsis citri* H. Fawcett non (Sacc.) Traverso & Spessa) (Whiteside and Timmer, 2000) are serious threats to production and marketability of fresh market citrus.

Citrus trees are often large and dense. This complicates adequate deposition on difficult-to-reach inner canopy leaves and fruit. Hence, fruit and foliar diseases are currently being controlled by regular fungicide spray applications at spray volumes ranging from 9000 to 16000 L ha^{-1} (medium to full cover sprays, respectively) in citrus-producing areas of South Africa. These methods of application provide an acceptable balance between efficacy and efficiency based on existing economic considerations (Grout, 1997, 2003). Spray application is a complex procedure due to the large number of contributing factors influencing spray deposition. Major influences on spray deposition efficiency and efficacy include canopy geometry and density (Jejčič *et al*., 2011), environmental conditions (Salyani, 2005, 2006), the use of appropriate machinery (Cooke and Hislop, 1993; Cunningham and Harden, 1998a, 1998b, 1999; Furness *et al*., 2006b; Salyani, 2005, 2006), spray technique (Salyani and Whitney, 1990; Furness *et al*., 1998; Salyani and Farooq, 2004), spray volume (Hoffmann and Salyani, 1996; Cunningham and Harden, 1999; Fourie *et al*., 2009), the fungicide or pesticide used (Sundaram and Sundaram, 1987; Zabkiewicz, 2007), the influence of adjuvants (Butler-Ellis *et al*., 1997; Gent *et al*., 2003; Green and Beestman, 2007; van Zyl *et al*., 2010a, 2010b), and the complex interaction between these factors (Whitney *et al*., 1988; 1989; Salyani, 1994, 2005, 2006; Stover *et al*., 2002b; Grout, 2003). Effective deposition of the active ingredient on the target surface (citrus leaves, twigs or fruit) is needed for effective disease control since disease control and spray deposition are directly related (Holownicki *et al*., 2002).

Inadequate spray deposition is the most common reason for treatment failures. Inadequate deposition is due to a number of factors of which run-off and the use of poor equipment and technique are amongst the most common (Salyani, 1994; Grout, 1997, 2003; Stover *et al*., 2002b; Fourie *et al*., 2009). Cunningham and Harden (1998a, 1998b, 1999) showed that spraying mature citrus trees with application volumes above 2000 L ha^{-1} is inefficient since the amount retained by trees decreases rapidly at spray volumes above 2000 L ha^{-1}. It was estimated from laboratory experiments on navel leaves and confirmed in field trials on 5 × 5 m mandarin trees that these mature citrus trees can retain about 2300 L ha^{-1} only (Cunningham and Harden, 1998b). Thus, a large proportion of higher spray volumes are lost due to run-off and exo- and endo-drift (Salyani and Farooq, 2004). Off-target application of fungicides and/or pesticides is not only an economic loss, but also a potential environmental problem (Salyani, 1994; Stover *et al*., 2002a; Salyani and Farooq, 2004; Furness *et al*., 2006a, 2006b). Given the history of reliance on high-volume spray application in South African citrus

production, research on the optimisation of spray application is urgently needed. The use of lower volume spray applications (Cunningham and Harden, 1999), adding adjuvants to spray mixtures (van Zyl *et al*., 2010a, 2010b) and increasing treatment concentrations are possible means to optimise fungicide deposition and reduce fungicide losses through run-off and drift in citrus (Cunningham and Harden, 1999; Salyani and Farooq, 2004).

Various methodologies for the evaluation of spray deposition effectiveness have been developed for a range of crops. Methods of evaluation range from relatively simple to more advanced methods. These include qualitative visual assessment of spray deposition on sprayed targets through the use of fluorescent tracers (Salyani and McCoy, 1989; Holownicki *et al*., 2002; Furness *et al*., 2006a, 2006b) and the use of droplet rating charts to evaluate deposition on actual or artificial targets (Holownicki *et al*., 2002; Furness *et al*., 2006a) These methods are relatively simple but lack the ability to accurately measure deposition quantity and quality since it is dependent on human discretion (Salyani and Whitney, 1988; Jiang and Derksen, 1995). More advanced methods for determining deposition quantity include chemical residue recovery techniques such as gas chromatography or atomic absorption, spectrophotometry of metals and nutrients (Ware *et al*., 1969; Yates *et al*., 1974; Byers *et al*., 1984) and also recovering sprayed fluorescent tracers from artificial and plant surfaces through washing techniques and determining deposition through fluorometry and colorimetry (Lake, 1988; Salyani and Whitney, 1988, 1990). These methods lack the ability to quantify the quality of coverage, such as uniformity of spray coverage on the target surface (Juste *et al*., 1990). Spray deposition measurement, specifically in terms of quantity and quality, was greatly improved through the development of deposition assessment protocols that combines fluorometry, digital photomicrographic imaging and digital image analysis (Hoffmann and Salyani, 1996; Brink *et al*., 2004, 2006; Fourie *et al*., 2009; van Zyl *et al*., 2010a, 2010b).

The objectives of this study were first to describe a novel deposition assessment protocol for the assessment of spray deposition quantity, quality and uniformity, based on previously described methods (Brink *et al*., 2004, 2006; Fourie *et al*., 2009; van Zyl *et al*., 2010a, 2010b), specifically for use in citrus spray application research; and second, to determine deposition benchmarks indicative of biologically effective deposition quantities. These benchmarks should allow for better interpretation of spray deposition results. For the latter objective, control of ABS with copper oxychloride was used as model system. ABS is an economically important disease of leaves, fruit and twigs of susceptible mandarin or tangerine (*Citrus reticulata* Blanco) and tangerine × grapefruit (*C. reticulata* × *C. paradisi* Macfad.) hybrids in many citrus-producing regions of the world (Kiely, 1964; Whiteside, 1976; Solel, 1991; Schutte *et al*., 1992; Timmer *et al*., 1998, 2003; Timmer, 2000; Vicent *et al*., 2000; Akimitsu *et al*., 2003; Elena, 2006; Reis *et al*., 2006). The causal agent of ABS is the necrotrophic tangerine pathotype of *Alternaria alternata* (Fr: Fr) Keissl., which produces the host selective/specific ACT-toxin

(Kohmoto *et al*., 1979, 1991, 1993; Solel, 1991; Timmer *et al*., 2003; Lin *et al*., 2009). The control of ABS relies mainly on preventative fungicidal sprays (Swart *et al*., 1998). This is also the case for citrus diseases such as CBS (Schutte *et al*., 1997; Kotzé, 2000) and melanose (Whiteside and Timmer, 2000). Like *A*. *alternata*, causal agents of these diseases infect at average temperatures between 22 and 27°C and wetness periods of *c.* 12 hours (Canihos *et al*., 1999, Timmer *et al*., 2000), making ABS a good model pathosystem for the purpose of this study.

MATERIALS AND METHODS

Spray deposition assessment protocol

Fluorescent pigment

The physical suitability of a yellow fluorescent pigment [South Australian Research and Development Institute (SARDI) Yellow Fluorescent Pigment, 40% EC (SARDI, Loxton, South Australia); 1 mL L^{-1}] as tracer for a contact copper fungicide [Villa Copper Oxychloride, 85% WP (Villa Crop Protection SA, Kempton Park, South Africa); copper oxychloride with 50% metallic copper equivalent; 2 g L^{-1})] was studied by comparing particle size. This was done together and separately at ×400 and ×1000 magnification (Nikon Eclipse E600 microscope; www.Nikon.com). Digital photographs (15 photographs for each particle type) were taken at both magnifications (Nikon DXM1200C; www.Nikon.com) mounted on the microscope using image capturing software (Nikon NIS elements imaging software F version 3.00SP7; www.nis-elements.com). The photos were stored in Exif-TIFF file format (image size 1372×1024 pixels) for subsequent image analysis (Image Pro Plus software version 6.2; www.mediacy.com). In total, 176 particles of each particle type were measured to determine mean diameter (average length of diameters measured at 2 degree intervals passing through measured particle's centroid; µm), each image calibrated accurately to scale of magnification. The concentration (mL^{-1}) of fluorescent or copper oxychloride particles was determined using a haemocytometer. Six haemocytometer counts were done for each particle type separately from three yellow fluorescent pigment and three copper oxychloride agitated suspensions (1 l); two counts from each solution as replications. All counts were done manually at ×400 magnification (Zeiss Axioskop; www.Zeiss.com). An ultra-violet light source (UV-A at ≈ 365 nm; Labino Mid-Light; www.labino.com) was used to illuminate the fluorescent particles.

Deposition benchmarks indicating effective disease control

Leaves

ABS-susceptible 'Nova' mandarin hybrid (*Citrus reticulata* Blanco; hybrid of clementine 'Fina' and tangelo 'Orlando') trees were grown in 10-l plastic pots in a glasshouse at 27°C. Drip irrigation and a monthly application of slow release fertilizer (3:1:2 of N:P:K) were used to

maintain the plants. The trees were regularly pruned to stimulate young growth (flush) production for use in experiments and too keep the trees small.

Inoculum

An isolate of *A. alternata* was recovered from symptomatic mandarin leaves in Nelspruit (Mpumalanga province, South Africa). It was single-spored and thereafter identified using conidium morphology as *A. alternata*. Pathogenicity tests on susceptible 'Nova' mandarin leaves confirmed it to be the tangerine pathotype of *A. alternata* (Whiteside, 1976). It was stored in the Stellenbosch University culture collection (STE-U no. 6592-6593). Single spore isolates were placed on potato dextrose agar (PDA; MERCK Biolab, Gauteng, South Africa) plates and incubated at 27°C for 7 to 14 days under 12-h light-dark cycle until abundant conidia were observed. Conidial suspensions were produced by pouring sterile water onto the PDA cultures and rubbing the surface gently with an L-shaped glass rod. The conidial suspension was filtered through two layers of cheesecloth and adjusted to 1×10^5 spores mL^{-1} with the use of a haemocytometer. To prevent the loss of fitness of the isolate, it was regularly inoculated and re-isolated from nontreated 'Nova' mandarin leaves.

Spray application

Ten to 15 young flush shoots were cut from 'Nova' mandarin trees in the glasshouse. Upper leaf surfaces of fresh detached young 'Nova' leaves [smallest: 2 to 3 days old, ±15×8 mm; largest: 7 to 10 days old, ± 55×30 mm] were sprayed by means of a gravity feed mist spray gun (ITW DEVILBISS Spray Equipment Products, USA) with a fluid nozzle tip of 1.5 mm in diameter mounted on a spray frame (steel framework 800×1410×660 mm). A single leaf was positioned on a wire mesh tray (angled at 30° to the bench top), while the spray gun was mounted at a distance of 600 mm away aiming squarely at the target. A pre-run-off spray volume of 0.5 mL of copper oxychloride (2 g L^{-1}) + fluorescent pigment (1 mL L^{-1}) and deionised water was sprayed at different concentrations: 0 [control treatment], 0.1, 0.2, 0.4, 0.6, 0.8, 1, 1.5 and 2 times the recommended concentration at a pressure of 185 kPa from an air compressor (Balma® 50 l; 1.5 kW; www.balma.com), custom-fitted with activated carbon filters to remove any possible oil contamination. The spray gun was cleaned with 70% ethanol solution, flushed with distilled water and air-dried after each treatment. The wire mesh trays were cleaned with 70% ethanol solution between treatments. Eight leaves were sprayed separately per concentration as experimental units. Treated leaves were then carefully placed, unsprayed side facing down, on water soaked paper towels inside plastic containers (300×60×250 mm).

Deposition analysis

Sprayed leaves were transported to a dark room in the plastic containers. A single leaf was positioned in the middle of a back-illuminated red Perspex box (300×210×110 mm) to reduce any shadowing and to enhance edging of leaves in captured images during analysis. The leaf was covered with a glass pane (200×200×2 mm) and illuminated using an ultra-violet light source (UV-A at ≈ 365 nm; Labino Mid-Light; www.labino.com). Digital photos were taken in Canon RAW file format (.CR2 ≈ 10 MB) of the upper leaf surfaces using a Canon EOS 40D camera equipped with a 60 mm macro lens. The camera was attached to a tripod in a fixed position directly above the leaf. RAW image files were converted to 8-bit Exif-TIFF files (.TIF ≈ 30 MB) with Digital Photo Professional version 3.1.0.0 (CANON INC.; www.canon.com) for digital image analysis (Image Pro Plus software version 6.2; Media Cybernetics, www.mediacy.com) to determine the deposition quantity and quality of the fluorescent particles per leaf.

Similar to the methodology used in Brink *et al*. (2004), Fourie *et al*. (2009) and van Zyl *et al*. (2010a; 2010b), deposition quantity per leaf was measured as percent total leaf area covered by pigment particles (percentage fluorescent particle coverage; FPC%). For the deposition quality assessment, the leaf area was divided into equally-sized squares of 150×150 pixels (22500 pixels). Depending on the leaf size, this amounted to as few as 10 to more than 110 individual squares per leaf, of which the percent area covered by fluorescent pigment particle was determined for each square. The coefficient of variation of the mean value of all the blocks analyzed per leaf (CV% = Standard Deviation × 100 / Mean) was used as a measure of deposition quality per leaf, *i.e.* uniformity of deposition on the leaf surface. Low CV% values were indicative of better deposition quality.

Inoculation with Alternaria alternata

Following deposition analysis, sprayed leaves were placed back into the containers and transported back to the spray chamber. This was usually 3-4 hours after spray application. Upper leaf surfaces of sprayed leaves were spray inoculated with pre-run-off volumes (0.3 mL) of 1×10^5 spores mL^{-1} suspension of *A. alternata*. Spray inoculation was done in the same manner described for spray application. The spray-inoculated leaves were placed on moistened paper towels and incubated in the plastic containers at high relative humidity (>95%) at 27°C in the dark for ≈48 h until pin-point necrotic lesions (< 2 mm in diameter) developed on the control treatment leaves.

Disease severity evaluation

After the incubation period, the leaves were removed from the plastic containers and the midrib of the leaves excised by means of a scalpel, splitting the leaves into two pieces. Leaf infection

was photographed before these lesions expanded and connected. Each piece was digitally photographed under white light on a white Perspex covered light box in exactly the same order as the leaves had been photographed previously for deposition analysis. Digital photographs of the symptomatic leaves were taken in JPEG (.JPG) format. Each photograph was manually analysed with Image Pro Plus software version 6.2 to determine the percentage symptomatic area per leaf. This was subsequently expressed as the relative percent disease control per leaf compared to the nontreated control treatment. After the leaves were photographed, they were stored by treatment in plastic bags at -20°C for copper residue analysis. The experiment was repeated 18 times.

Copper residue analysis

The 18 repetitions were grouped into three batches to allow sufficient biomass for copper residue analysis, which was done on each batch separately by an accredited analytical laboratory (SGS Analytical Laboratory, Somerset-West, South Africa). Briefly, analysis involved dry ashing of 1 g of plant material in a crucible, and digested (ashed) by heating in a muffle furnace (500°C for 4 h). The ash residue was then dissolved in 5 mL 6 N HCL and 6 N HNO_3 mixture, diluted to 100 mL with distilled water, filtered and copper ionic particle residue determined from 25 mL (each sample) as mg kg^{-1} by inductively coupled plasma (ICP) spectrometer (Perkin-Elmer AAnalyst 400; www.perkinelmer.com).

Statistical analyses

Particle size, number of particles mL^{-1}, deposition quantity (FPC%) and quality (CV% per leaf), copper residue and percentage disease control (actual and predicted) data were subjected to appropriate analysis of variance (ANOVA). Fisher's LSD was calculated to identify significant differences between treatments at a 95% confidence interval. Data were also subjected to Pearson's correlation was used to demonstrate the linear relation between treatment (concentrations) and copper residue, deposition quantity and quality measurements. All statistical analyses were done using software Addinsoft XLSTAT Version 2011.2.06 (www.xlstat.com).

Benchmark modelling

For benchmark modelling, various functions (natural growth functions, allometric, Gompertz and Hoerl) were evaluated to model the relationship between deposition quantity (FPC%) and ABS control by fitting the selected data to the functions through iterative non-linear least square regression using the NLIN procedure-modified Gauss-Newton method of SAS Version 8.2 statistical analysis software. Pearson's correlation between actual and predicted values was used to evaluate goodness of fit, from which the best model was selected. Proportion of

Variance Explained (%PVE) was used to evaluate goodness of fit. Two benchmarks, FPC_{50} and FPC_{75}, were selected, which indicate deposition quantity levels that would result in a predicted 50% and 75% disease control, respectively.

Protected leaf area was determined by expressing the number of blocks (as obtained in the deposition quality analysis) that had an FPC% value above that of the FPC_{50} benchmark as a percentage of the total number of blocks per leaf. This was also indicative of the quality of coverage per leaf.

RESULTS

Spray deposition assessment protocol

Fluorescent pigment

Microscopic observation of the fluorescent and copper oxychloride particles indicated distinct differences in particle shape and colour. The more abundant, smaller, solid, sharp edged, light green fluorescent particles could easily be differentiated from the larger, rigid, granular, dark grey copper oxychloride particles in suspension. Analysis of variance (ANOVA) indicated a significant difference (P = 0.004) in the mean diameter (µm) between the two particle types with copper oxychloride particles having a larger average mean diameter (3.89 µm) than the fluorescent pigment particles (3.31 µm; Table 1). Likewise, there was significantly (P = 0.007) fewer particles mL^{-1} counted for the copper oxychloride (3.73×10^7 particles mL^{-1}) than for the pigment particles (4.26×10^7 particles mL^{-1}; Table 1).

Deposition benchmarks indicating effective disease control

Spray application

A distinct droplet pattern could be observed on leaves following the addition of yellow fluorescent pigment to the spray mixture when illuminated with black light. Droplets formed separately in a distinct deposition pattern that occasionally connected, forming larger elongated droplets on leaf surfaces (Fig. 1). The presence and aggregation of pigment particles inside the spray deposit varied with different treatment concentrations. The fluorescent pigment particle residue in the dried spray deposit made droplet formation visible on the leaf surface. The illumination of formed spray deposit on sprayed leaf surfaces varied in intensity as the concentration of fluorescent pigment varied per spray treatment. Light reflectance of formed spray deposit was most intense and visible at the highest spray treatment concentration of 2× copper oxychloride and fluorescent pigment with intensity decreasing linearly to a point of least visible visual observation at the lowest spray treatment concentration of 0.1× copper oxychloride and fluorescent pigment. On the control treatments, no fluorescent pigment was observed as no fluorescent pigment was added.

Deposition analysis

ANOVA of deposition quantity (FPC%) and deposition quality (CV%) data indicated significant effects for copper oxychloride concentration (treatments) ($P < 0.0001$). From Pearson's correlation it was shown that deposition quantity increased linearly with increase of treatment concentrations ($r = 0.879$). Deposition quantity (FPC%) values ranged from 0.86% at 0.1× to 12.39% at 2× concentration (Table 2). There was a decrease in CV% values (*i.e.* improved deposition quality) as the treatment concentrations increased (36.40% at 0.1× to 24.24% at 2× concentration), but deposition quality did not differ significantly for concentrations from 0.4 to 2 (Table 2).

Disease severity evaluation

Very small brown to black lesions (0.5 to 2 mm) with white to yellow halos were observed on the spray-inoculated leaf surfaces after ≈48-h incubation. Leaf infection was photographed before these lesions expanded and connected. From visual observation, it was clear that as copper oxychloride concentration increased, the number and size of infection points decreased. ANOVA of disease control data (%) indicated significant treatment effects ($P < 0.0001$). The percentage disease control improved linearly ($r = 0.743$) from 37.22% to 92.94% as the treatment concentration increased (Table 3).

Copper residue analysis

ANOVA of Cu-residue data (mg kg^{-1}) indicated significant treatment effects ($P < 0.0001$). As the treatment concentrations increased, the Cu residue levels increased with a very good linear fit ($r = 0.992$). The highest residue level was obtained following the highest treatment concentration 2× (239.512 mg kg^{-1}) and the lowest level on the control treatment (7.80 mg kg^{-1}), which is indicative of the inherent Cu content of the sprayed leaves (Table 3). Pearson's correlation indicated a very good linear relationship between Cu residue levels and deposition quantity (FPC%) ($r = 0.851$) and between Cu residue levels and the disease control achieved ($r = 0.748$).

Benchmark modelling

Various models were fitted for deposition quantity and disease control data with a 'von Bertalanffy growth function' with the asymptote set at 100 (*i.e.* maximum disease control that can theoretically be achieved) fitting the data best: *E[Disease control(%)] = 100[1 – exp(-0.3346(% FPC))]* (91.04 %PVE) with a good correlation between observed and predicted values ($r = 0.825$) (Table 3). The 95% confidence limits for the *b*-value, -0.3346, were -0.3741 to -0.2951.

Following the curvature of the model (Fig. 2), predicted disease control increased as the deposition quantity (FPC%) increased. The slope of line in the initial stage of the graph (0 to 2 FPC%) was very steep, indicating a very high proportional contribution to disease control (*c.* 50%). The slope declined as the deposition quantity increased (2 to 4 FPC%), indicating a more moderate proportional contribution to disease control (*c.* 25%), and declined further toward the asymptote, with the effect of increasing deposition quantity on disease control declining proportionally (4 to 6 FPC% added *c*. 12.5% disease control and 6 to 8 FPC% added *c.* 6.5% disease control). The FPC_{50} and FPC_{75} benchmarks indicating a predicted 50% or 75% disease control were calculated from the model as 2.07 FPC% and 4.14 FPC%, respectively.

ANOVA of percent protected leaf area indicated significant effects for treatments ($P < 0.0001$). Protected leaf area was positively correlated with treatment concentration ($r = 0.879$). Means of actual deposition quantity (FPC%) data were used in the FPC benchmark model to calculate the predicted disease control following the various treatments (Table 3). Pearson's correlation between the actual and predicted disease control (FPC%) was very good ($r = 0.826$).

DISCUSSION

This study describes an improvement on a previously described spray deposition assessment protocols (Brink *et al*., 2006; Fourie *et al*., 2009; van Zyl *et al*., 2010a, 2010b) and provides new information on the suitability of a fluorescent pigment that has been used as tracer in these and other studies (Furness *et al*., 2006b). Additionally, by using control of ABS of mandarins with copper oxychloride as a model system, this study models fluorescent pigment deposition benchmarks indicative of effective disease control. These benchmarks are important in the biological interpretation of spray deposition.

The yellow fluorescent pigment was shown to be an ideal tracer based on its particle physical characteristics in comparison with the contact fungicide copper oxychloride. Although not statistically similar, the particle types of these compounds are closely related in mean diameter and in formulation and recommended concentrations for use had similar amounts of particles. Prior unpublished studies have also shown that the suspension concentrate formulation of the yellow fluorescent pigment had minimal effects on water droplet characteristics on leaf surfaces (unpublished results). Cooke and Hislop (1993) and Palladini *et al*. (2005) showed the importance of choice of fluorescent tracer, specifically that it must be visualized when dry on a target surface and be photo-stable. Previous studies with the yellow fluorescent pigment did not investigate photo-stability but did show the effectiveness of using the tracer under various conditions, its adherence to the target surfaces and ease of visualisation once the pigment has dried. Recently, Schutte *et al*. (2012) clearly showed the

effectiveness, photo-stability and persistence of the yellow fluorescent pigment over a period of 6 weeks. Other commonly used water-soluble fluorescent tracers (sodium salt of fluorescein) degraded by 20% after 30 min if exposed to direct sunlight on artificial surfaces (e.g. water-sensitive paper). Thus, sampling must occur as soon as possible after application and stored in light proof containers. This reduces the size of trial layouts and amount of sample material that can be used since sampling must be done as soon as possible (Holownicki *et al*., 2002).

Previously, Brink *et al*. (2004, 2006), Fourie *et al*. (2009) and van Zyl *et al*. (2010a, 2010b) used high magnification photomicrography of specific areas of sprayed target surfaces. This method required specialized light sources and stereomicroscopes and was extremely time consuming. Criticism was also levelled at this technique in that it focussed on deposition on small target surfaces, and to some extent ignored the general deposition trends in the canopy since only smaller number of samples could be analysed. Image capturing methodology was changed to photomacrography of whole target surfaces, i.e. leaves or fruit. This enables spray deposition research on biological targets in natural environments. This is more accurate than the use of artificial targets that are sometimes used to simulate deposition on natural targets (Koch and Knewitz, 2008). The high-quality digital images (8-bit Exif-TIFF (.TIF ≈ 30 MB) allowed for clear visualization, measurement and calculation of the quantity and quality of particle deposition on the target surfaces, even very small pigment particles on the target surface that could not be observed through the use of the image analysis software in previous protocols. For example, earlier comparisons with this technology and water sensitive paper showed pigment particles deposited on the paper where the amount of liquid in the droplet was not enough to induce colour change (results not shown). Additional changes to digital image analysis included improved image binarization, contrast and colour enhancement, and proprietary scripting of macros for quantity and quality analyses in Image Pro-Plus, which further improved the ease, accuracy and sensitivity of the deposition quantity and quality measurements.

The aforementioned changes improved the efficiency and ‘user-friendliness’ of the deposition assessment protocol allowed for considerably improved throughput of sample analysis. Hence, spray deposition analysis of effects such as deposition quantity and quality on multiple target surfaces (for example, upper and lower leaf surfaces), as well as uniformity between target surfaces and spatial deposition in canopies could accurately be determined. Van Zyl *et al.* (2010a, 2010b) demonstrated the superior sensitivity of the photomicrography and fluorometry deposition assessment protocol over that of Furness *et al*. (2006a). The photomacrography and fluorometry deposition assessment protocol developed in the present study was not evaluated against other relevant deposition assessment protocols, but the

excellent correlation between deposition quantity measurements and copper residues and control of ABS should bear sufficient testament of its efficacy and robustness.

In the benchmark experiments, we observed very good positive linear correlation between copper oxychloride concentration, deposition quantity (FPC%), Cu residue and ABS control measured on the leaf surfaces. This supports the accuracy of the benchmark model as well as the suitability of yellow fluorescent pigment as tracer for copper oxychloride, and most probably other contact fungicides.

The FPC_{50} and FPC_{75} benchmarks were obtained from a 'von Bertalanffy growth function' fitted to 2592 deposition quantity *vs.* disease control data points, making the model sufficiently robust. The model predicts the deposition quantities needed for 50% and 75% disease control as 2.07 and 4.14 FPC%, respectively. Thus, the predicted copper oxychloride concentration needed for 50% and 75% disease control would be equivalent to 0.34× and 0.68× of the current registered concentration (200 g 100 L^{-1}), respectively. However, these benchmarks are built on efficacy data generated following inoculation on the day of fungicide application, and do not account for weathering, wash-off and residue breakdown. Vicent *et al*. (2009) evaluated rain fastness and protectant activity of copper fungicides against ABS at lower concentrations and found effective and similar disease control with copper oxychloride (50% metallic copper) at 0.25× and 0.5× of the recommended registered concentration (200 g 100 l^{-1}) over a 28-day period. Based on FPC benchmark model predictions, these concentrations would have realized 39.90% and 63.89% disease control, respectively. This indicates that field deposition quantities higher than the FPC_{50} value might be sufficient for ABS control under normal disease pressure conditions, and that deposition quantities *circa* the FPC_{75} value might be needed under high disease pressure conditions.

The benchmark model further indicates that very high deposition quantities (above FPC_{75} value) appear not to contribute significantly to decay disease control and future use of these benchmarks in orchard spray deposition assessment might indicate cases of over application and potentially reduced agrochemical use. This in turn will reduce the risk of phytotoxicity (Albrigo *et al*., 1997), stippling burn (Schutte *et al*., 1997) and environmental pollution (Alva *et al*., 1993) induced by excessively high spray volumes or high concentrations of contact copper fungicides. Further field validation of the benchmark values would, however, be required to support such recommendations.

Deposition quality data were not used in the construction of the model, as the spray methods used (pre-run-off sprays at set spray volumes) attempted and succeeded to minimize deposition quality differences between treatments. Hence, the deposition quality dataset did not allow sufficient variation between treatments to be incorporated in the model. As deposition quality undoubtedly influence efficacy (Koch and Knewitz, 2008; Fourie *et al*., 2009, 2011; van Zyl *et al*., 2010b), future research will attempt to include deposition quality measurements in

the FPC benchmark model. However, the model was effective in determining the percentage protected leaf area above the FPC_{50} benchmark. This parameter can therefore also used as a deposition quality indicator.

The FPC benchmark model can be an effective tool to evaluate deposition of varying spray volumes, spray machines and technique for the control of ABS and similar fruit and foliar diseases. Fungicide dosage/concentration can also be evaluated leading to implementation of effective but environmentally sound application rates.

ACKNOWLEDGEMENTS

The authors acknowledge Citrus Research International and THRIP for funding and Marieta Van Der Rijst (ARC-Biomet, Stellenbosch) for insight into statistical data modelling. The first author also acknowledges the National Research Foundation, Bayer CropScience South Africa and the Department of Plant Pathology, Stellenbosch University for bursary funding.

REFERENCES

Akimitsu, K., Peever, T.L., Timmer, L.W., 2003. Molecular, ecological and evolutionary approaches to understanding *Alternaria* diseases of citrus. Molecular Plant Pathology 4: 435-446.

Albrigo, L.G., Timmer, L.W., Townsend, K., Beck, H.W., 1997. Copper fungicide residue for disease control and potential spray burn. Proceedings of the Florida State Horticultural Society 110: 67-70.

Alva, A.K., Graham, J.H., Tucker, D.P.H., 1993. Role of calcium in amelioration of copper phytotoxicity for citrus. Soil Science 155: 211-218.

Brink, J.C., Calitz, F.J., Fourie, P.H., Holz, G., 2004. Development of a protocol to quantify spray deposits on grape bunches. 7th International Symposium on Adjuvants for Agrochemicals ISAA 2004, ISAA 2004 Foundation, South Africa, 230-236.

Brink, J.C., Fourie, P.H., Holz, G., 2006. Effect of fungicide spray cover on *Botrytis cinerea* infection in grape bunches. South African Journal of Enology and Viticulture 27: 51-56.

Butler Ellis, M.C., Tuck, C.R., Miller, P.C.H., 1997. The effect of some adjuvants on sprays produced by agricultural flat fan nozzles. Crop Protection 18: 41-50.

Byers, R.E., Barden, J.A., Donohue, S.J., Horsburgh, R.L., Lyons jr., C.G., Yoder, K.S., 1984. Effects of apple tree size and canopy density on spray chemical deposit. HortScience 19: 93-94.

Canihos, Y., Peever, T.L., Timmer, L.W., 1999. Temperature, leaf wetness, and isolate effects on infection of Minneola tangelo leaves by *Alternaria* sp. Plant Disease. 83: 429-433.

Cooke, B.K. and Hislop, E.C. 1993. Spray tracing techniques. Pages 85-100 in: Application technology for crop protection. Matthews, G.A., Hislop, E.C. Wallingford: CAB.

Cunningham, G.P., Harden, J., 1998a. Air-tower sprayers increase spray application efficiency in mature citrus trees. Australian Journal of Experimental Agriculture. 38: 871-877.

Cunningham, G.P., Harden, J., 1998b. Reducing spray volumes applied to mature citrus trees. Crop Protection 17: 289-292.

Cunningham, G.P., Harden, J., 1999. Sprayers to reduce spray volumes in mature citrus trees. Crop Protection 18: 275-281.

Elena, K., 2006. Alternaria brown spot of Minneola in Greece; evaluation of citrus species susceptibility. European Journal of Plant Pathology 115: 259-262.

Fourie, P.H., Brink, J.C., du Preez, M., Schutte, G.C., 2009. The effect of runoff on spray deposition and control of Alternaria brown spot of mandarins. Australasian Plant Pathology 38: 173-182.

Furness, G.O., Drew, H.J., Magarey, P.A., Miller, P.H., 1998. Fruit tree and vine sprayer calibration based on canopy size and length of row: Unit canopy row method. Crop Protection 17: 639-644.

Furness, G.O., Manktelow, D.W.L., Thompson, A.J., 2006a. A Visual droplet number rating chart and fluorescent pigment sprays to estimate chemical deposition and spray coverage on plant foliage. Aspects of Applied Biology: 77: 171-178.

Furness, G.O., Manktelow, D.W.L., Thompson, A.J., 2006b. Multi-fan spray towers to improve dose efficiency and spray coverage uniformity in citrus trees. Aspects of Applied Biology 77: 481-488.

Gent, D.H., Nissen, S.J., Schwartz, H.F., 2003. Effect of commercial adjuvants on vegetable crop fungicide coverage, absorption and efficacy. Plant Disease 87: 591-597.

Green, J.M., Beestman, G.B., 2007. Recently patented and commercialized formulation and adjuvant technology. Crop Protection 26: 320-327.

Grout, T.G., 1997. Spray volumes and coverage requirements for citrus in South African Citrus Journal 31: 19-20.

Grout, T.G., 2003. Use of plant protection products. Section 2.7 in: Citrus production guidelines-Volume III. Integrated Pest Management. (Ed. T G Grout) (Citrus Research International: Nelspruit, South Africa).

Hall, F.R., Cooper, J.A., Ferree, D.C., 1991. Orchard geometry and pesticide placement. BCPC Monographs 46: 171-176.

Holownicki, R., Doruchowski G., Jaeken, P., Świechowski, W., 2002. Methods of evaluation of spray deposit and coverage on artificial targets. Electronic Journal of Polish Agricultural Universities (EJPAU) 5(1), #03. Online publication.

Jejčič, V., Godeša, T., Hočevar, M., Širok, B., Malneršič, A., Štancar, A., Lešnik, M., Stajnko, D., 2011. Design and testing of an ultrasound system for targeted spraying in orchards. Strojniški vestnik – Journal of Mechanical Engineering 57: 587-598.

Jiang, C., Derksen, C.R., 1995. Morphological image processing for spray deposit analysis. Transactions of the American Society of Agricultural Engineers 38: 1581-1591.

Juste, F., Sánchez, S., Ibáñez, R., Val, L., García, C., 1990. Measurement of spray deposition and efficiency of pesticide application in citrus orchards. Journal of Agricultural Engineering Research 46: 187-196.

Kiely, T.B., 1964. Brown spot of Emperor mandarin. The agricultural gazette of New South Wales 75: 854-856.

Koch, H., Knewitz, H., 2008. Methodology and sampling technique of spray deposit and distribution measurement in vineyards. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 60: 25-30.

Koch, H., Knewitz, H., 2011. Application quality – how to measure – how to interpret. CtiFl SuProFruit 11th Workshop, Bergerac, France, 8-10 June, 2011, Book of Abstracts, Online version.

Kohmoto, K., Scheffer, R.P., Whiteside, J.O., 1979. Host-selective toxins from *Alternaria citri*. Phytopathology 69: 667-671.

Kohmoto, K., Akimitsu, K., Otani, H., 1991. Correlation of resistance and susceptibility of citrus to *Alternaria alternata* with sensitivity to host-specific toxins. Phytopathology 81: 719-722.

Kohmoto, K., Itoh, Y., Shimomura, N., Kondoh, Y., Otani, H., Kodama, M., Nishimura, S., Nakatsuka, S., 1993. Isolation and biological activities of two host-specific toxins from the tangerine pathotype of *Alternaria alternata*. Phytopathology 83: 495-502.

Kotzé, J.M., 2000. Black spot. Pages 23-25 in: Compendium of Citrus Diseases (second Edition). Timmer, L.W., Garnsey, S.M. and Graham, J.H. APS Press, The American Phytopathological Society, St. Paul Minnesota, USA.

Lake, J.R., 1988. The deposition of electrostatically charged sprays on parts of targets shaded from the spray. Journal of Agricultural Engineering Research 39: 9-18.

Lin, C-H., Ling Yang, S., Chung, K., 2009. The YAP1 homolog-mediated oxidative stress tolerance is crucial for pathogenicity of the necrotrofic fungus *Alternaria alternata* in citrus. Molecular Plant-Microbe Interactions 22: 942-952.

Palladini, L.A., Raetano, C.G., Velini, E.D., 2005. Choice of tracers for the evaluation of spray deposits. Scientia Agricola 62: 440-445.

Reis, R.F., de Goes, A., Mondal, S.N., Shilts, T., Brentu, F.C., Timmer, L.W., 2006. Effect of lesion age, humidity, and fungicide application on sporulation of *Alternaria alternata*, the cause of brown spot of tangerine. Plant Disease 90: 1051-1054.

Salyani, M., 1994. Spray technology research for orchard applications. Acta Horticulturae 372: 67-74.

Salyani, M., 2005. 2005 Florida citrus pest management guide: Pesticide Application Technology. AE-259, series of the Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu.

Salyani, M., 2006. 2006 Florida citrus pest management guide: Pesticide Application Technology. ABE-356, series of the Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu

Salyani, M., Farooq, M., 2004. Drift potential of citrus air-carrier sprayers. Proceedings of the Florida State Horticultural Society 117: 130-135.

Salyani, M., Hoffmann, W.C., 1996. Effects of application time and spray volume on deposition. Proceedings of the Florida State Horticultural Society 109: 46-50.

Salyani, M., McCoy, C.W., 1989. Deposition of different spray volumes on citrus trees. Proceedings of the Florida State Horticultural Society 109: 46-50.

Salyani, M., Whitney, J.D., 1988. Evaluation of methodologies for field studies of spray deposition. Transactions of the American Society of Agricultural Engineers 31: 1680-1684.

Salyani, M., Whitney, J.D., 1990. Ground speed effect on spray deposition inside citrus trees. Transactions of the American Society of Agricultural Engineers 33: 361-366.

Schutte, G.C., 1996. First report of Alternaria brown spot on Star Ruby grapefruit in South African. Citrus Journal 6, 24.

Schutte, G.C., Lesar, K.H., Pelser, P. du T., 1992. The use of tebuconazole for the control of *Alternaria alternata* on 'Minneola' Tangelos and its potential to control postharvest decay when applied as a preharvest spray. Proceedings of the International Society of Citriculture 3: 1070-1074.

Schutte, G.C., Beeton, K.V., Kotzé, J.M., 1997. Rind stippling on Valencia oranges by copper fungicides used for control of citrus black spot in South Africa. Plant Disease 81: 851-854.

Schutte, G.C., Kotze, C., van Zyl, J.G., Fourie, P.H., 2012. Assessment of retention and persistence of copper fungicides on orange fruit and leaves using fluorometry and copper residue analyses. Crop Protection 42: 1-9.

Solel, Z., 1991. Alternaria brown spot on Minneola tangelos in Israel. Plant Pathology 40: 145-147.

Stover, E., McClure, P., Salvatore, J., 2002. Influence of spray volume, ground speed, and a sensor-actuated spray controller on melanose control and fruit marking in fresh Indian river grapefruit. Proceedings of the Florida State Horticultural Society 115: 1-5.

Stover, E., Scotto, D., Wilson, C., Salyani, M., 2002. Spray application to citrus: Overview of factors influencing spraying efficacy and off-target deposition. HS-851, series of the Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu.

Sundaram, K.M.S., Sundaram, A., 1987. Role of formulation ingredients and physical properties on droplet size spectra, deposition, and persistence of aerially sprayed amonicarb and mexacarbate in forest litter soil samples. ASTM STP 968: Pesticide Formulations and Application Systems 7: 139-151

Swart, S.H., Wingfield, M.J., Swart, W.J., Schutte, G.C., 1998. Chemical control of Alternaria brown spot on Minneola tangelo in South Africa. Annals of Applied Biology 133: 17-30.

Timmer, L.W., Solel, Z., Gottwald, T.R., Ibañez, A.M. and Zitko, S.E. 1998. Environmental factors affecting production, release, and field populations of conidia of *Alternaria alternata*, the cause of brown spot of citrus. Phytopathology 88: 1218-1223.

Timmer, L.W., 2000. Alternaria brown spot of Mandarins. Pages 19-20 in: Compendium of Citrus Diseases (second Edition). Timmer, L.W., Garnsey, S.M. and Graham, J.H. APS Press, The American Phytopathological Society, St. Paul Minnesota, USA.

Timmer, L.W., Darhower, H.M., Zitko, S.E., Peever, T.L., Ibanez, A.M., Bushong, P.M., 2000. Environmental factors affecting the severity of Alternaria brown spot of citrus and their potential use in timing fungicide applications. Plant Disease 84: 638-643.

Timmer, L.W., Peever, T.L., Solel, Z. And Akimitsu, K. 2003. Alternaria diseases of citrus - Novel pathosystems. Phytopathologia Mediterranea 42: 3-16.

van Zyl, S.A., Brink, J.C., Calitz, F.J., Fourie, P.H., 2010a. Effects of adjuvants on deposition efficiency of fenhexamid sprays to Chardonnay grapevine foliage. Crop Protection 29: 843-852.

van Zyl, S.A., Brink, J.C., Calitz, F.J., Coertze, S., Fourie, P.H., 2010b. The use of adjuvants to improve spray deposition and *Botrytis cinerea* control on Chardonnay grapevine leaves. Crop Protection 29: 58-67.

Vicent, A., Armengol, J., Sales, R., García – Jiménez, J., Alfaro – Lassala, F., 2000. First report of Alternaria brown spot of citrus in Spain. Plant Disease 84: 1044.

Vicent, A., Armengol, J., García-Jiménez, J., 2009. Protectant activity of reduced concentration copper sprays against Alternaria brown spot on 'Fortune' mandarin fruit in Spain. Crop Protection 28: 1-6.

Ware, G.W., Estesen, B.J., Cahill, W.P., Gerhardt, P.D., Frost, K.R., 1969. Pesticide drift. I. High-clearance vs. aerial application of sprays. Journal of Economic Entomology 62: 840-843.

Whiteside, J.O., 1976. A newly recorded *Alternaria*-induced brown spot disease on Dancy tangerines in Florida. Plant Disease Reporter 60: 326-329.

Whiteside, J.O., Tmmer, L.W., 2000. Melanose. Page 28-29 in: Compendium of Citrus Diseases (second Edition). Timmer, L.W., Garnsey, S.M. and Graham, J.H. APS Press, The American Phytopathological Society, St. Paul Minnesota, USA.

Whitney, J.D., Churchill, D.B., Knapp, J.L., Littell, R.C., Salyani, M., Whiteside, J.O., 1988. Ground speed and spray volume of airblast sprayers affect copper deposition and greasy spot control. Proceedings of the Florida State Horticultural Society 101: 13-17.

Whitney, J.D., Salyani, M., Churchill, D.B., Knapp, J.L., Whiteside, J.O., Littel, R.C., 1989. A field investigation to examine the effects of sprayer type, ground speed and volume rate on spray deposition in Florida citrus. Journal of Agricultural Engineering Research 42: 275-283.Yates, W.E., Akesson, N.B., Cowen, R.E., 1974. Criteria for minimizing drift residues on crops downwind from aerial applications. Transactions of the American Society of Agricultural Engineers 17: 627-632.

Zabkiewicz, J.A., 2007. Spray formulation efficacy-holistic and futuristic perspectives. Crop Protection 26: 312-319.

Table 1. Differences in mean particle diameter (µm) and number of particles mL^{-1} of suspensions of yellow fluorescent pigment (1 mL L^{-1}) and copper oxychloride (2 g L^{-1}).

	Measurements			
Particle type	**Minimum**	**Maximum**	**Mean[b]**	**Std. Deviation**
Mean diameter (µm)[a]				
Copper oxychloride	0.55	8.23	3.87 a	1.49
Pigment	0.70	9.15	3.31 b	2.12
Particles/mL				
Copper oxychloride	34.0×10^6	40.2×10^6	37.3×10^6 a	2.23×10^6
Pigment	38.4×10^6	45.9×10^6	42.6×10^6 b	3.07×10^6

[a] Average length of diameters measured at 2 degree intervals passing through the centroid of the measured objects

[b] For each parameter separately, values in each column followed by the same letter do not differ significantly ($P > 0.05$) according to Fisher's least significant difference test

Table 2. Deposition quantity (FPC%) and deposition quality [CV% and protected leaf area (%)], on young 'Nova' leaves sprayed with various concentrations of yellow fluorescent pigment (0 to 2× of 1 mL L^{-1}) and copper oxychloride (0 to 2× of 2 g L^{-1}).

	Deposition		
Treatment[a]	**Quantity**	**Quality**	
	(FPC%[c])[b]	**CV%[b]**	**Protected leaf area (%)[b]**
2.0	12.39 a	24.24 c	97.63 a
1.5	9.63 b	25.24 c	94.99 a
1.0	5.45 c	23.79 c	89.02 a
0.8	4.79 c	25.76 c	75.87 b
0.6	3.47 d	25.85 c	59.11 c
0.4	2.72 d	26.31 c	40.94 d
0.2	1.42 e	31.52 b	18.74 e
0.1	0.86 ef	36.40 a	3.97 f
0 (control)	0.00 f		0.00 f

[a] Factor of recommended application rate of copper oxychloride (2 g L^{-1}) and yellow fluorescent pigment (1 mL L^{-1})

[b] For each parameter separately, values in each column followed by the same letter do not differ significantly ($P > 0.05$) according to Fisher's least significant difference test

[c] Deposition quantity as expressed by percentage leaf area covered by fluorescent particles

Table 3. Mean Alternaria brown spot control (%), predicted disease control (%), and copper residue (mg kg^{-1}) determined on young 'Nova' mandarin leaves sprayed with various concentrations of yellow fluorescent pigment (0 to 2× of 1 mL L^{-1}) and copper oxychloride (0 to 2× of g L^{-1}) and subsequently spray-inoculated with *Alternaria alternata*.

Treatment[a]	Disease control [b]		Cu residue (mg kg^{-1})[b]
	Observed (%)	Predicted (%)[c]	
2.0	92.94 a	86.36 a	239.51 a
1.5	90.39 a	83.24 ab	189.94 b
1.0	75.49 b	76.26abc	146.44 c
0.8	70.13 bc	70.36 bc	109.03 d
0.6	63.34 cd	64.56 cd	87.33 e
0.4	56.45 d	56.41 d	60.54 f
0.2	44.29 e	36.91 e	31.07 g
0.1	37.22 e	23.93 f	16.07 h
0 (control)	0.00 f	0.00 g	7.80 h

[a] Factor of recommended application rate of copper oxychloride (2 g L^{-1}) and yellow fluorescent pigment (1 mL L^{-1})

[b] For each parameter separately, values in each column followed by the same letter do not differ significantly ($P > 0.05$) according to Fisher's least significant difference test

[c] Predicted control calculated by subjecting deposition quantity data to fluorescent particle coverage benchmark function: [Disease control = $100*^{(1-e\ (-0.3346*\ \%\ FPC)}$]

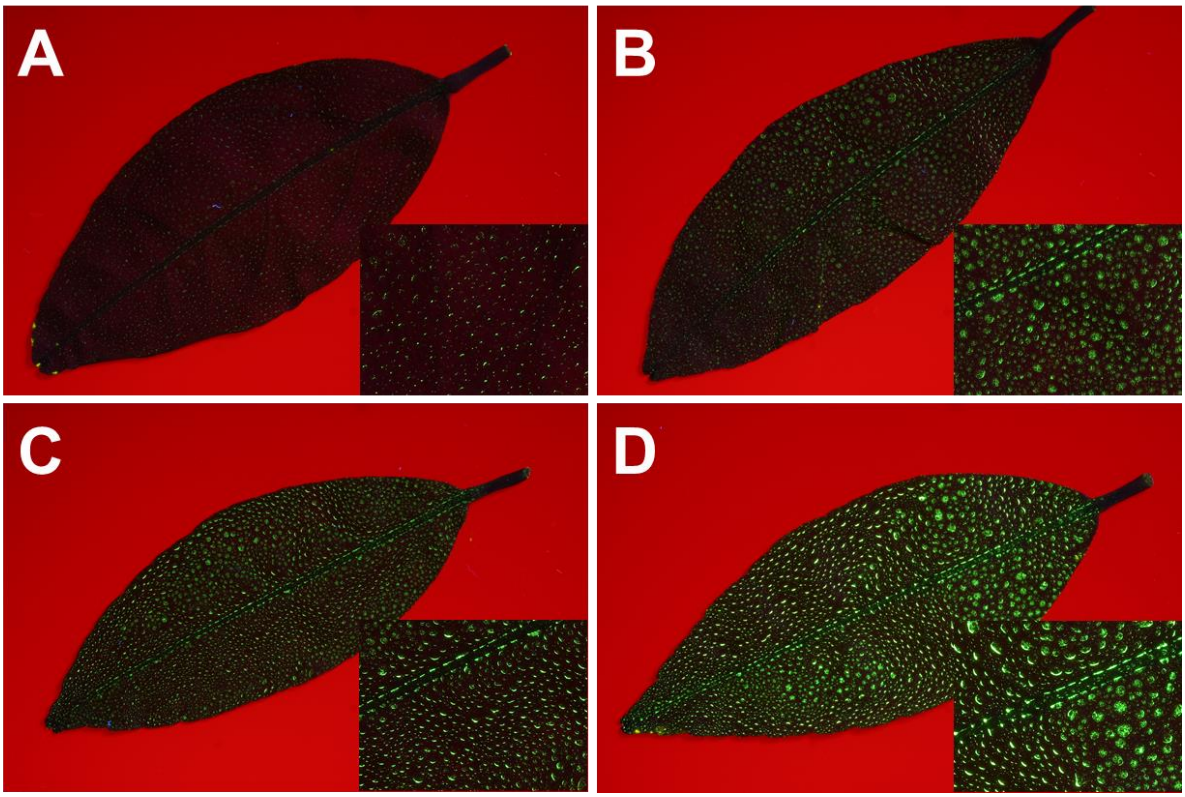

Figure 1. Digital images of the upper leaf surfaces of young 'Nova' mandarin leaves, and leaf sections at 2× digital zoom, that were illuminated with UV-A light illustrating the increase in treatment concentration (A = 0.1×; B = 0.8×; C = 1.0×; D = 2.0×) of copper oxychloride and a yellow fluorescent pigment.

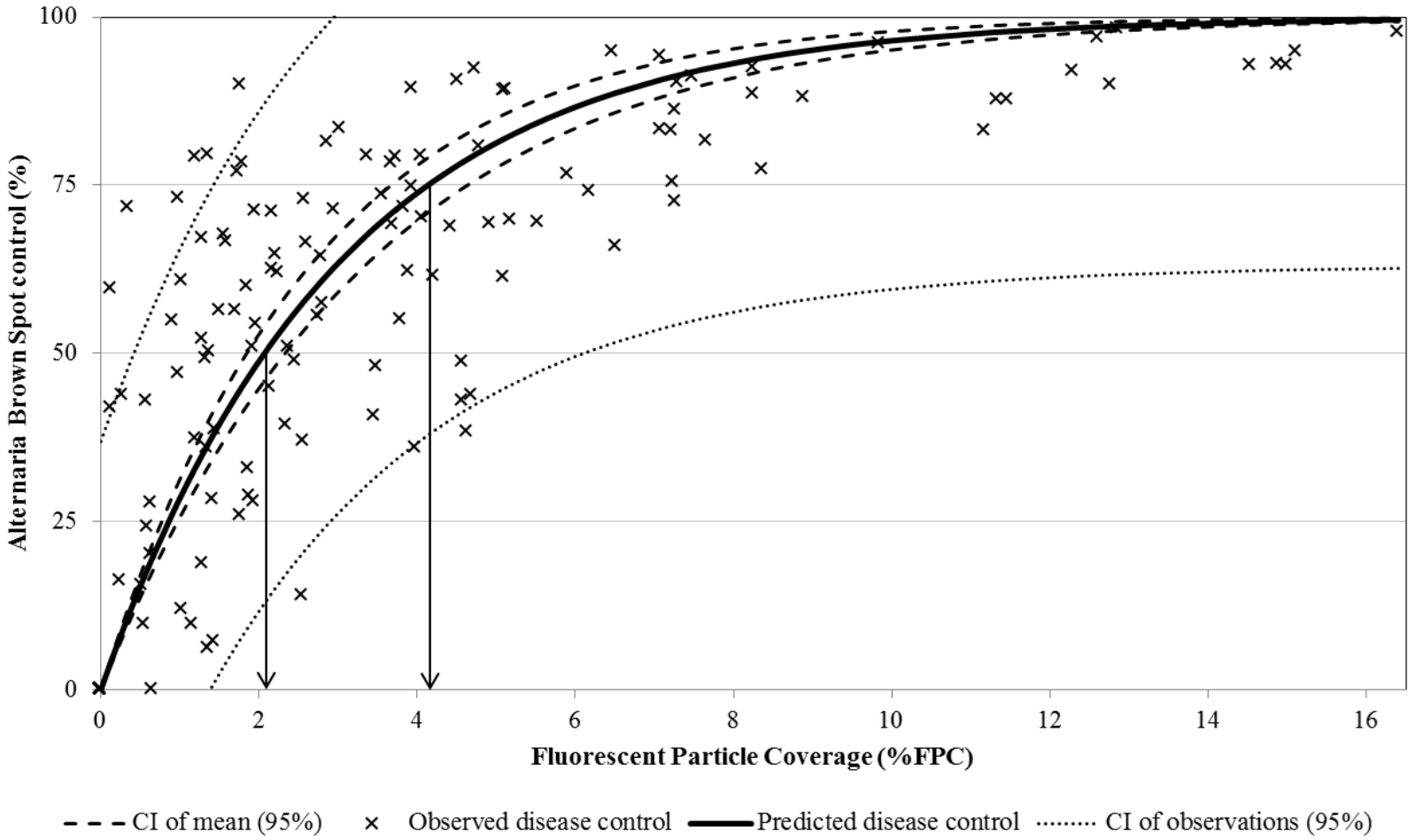

Figure 2. A von Bertalanffy model predicting Alternaria brown spot (ABS) control [Control = $100^{*(1-e^{(-0.3346*\%\ FPC)})}$] built on deposition quantity (FPC%) and concomitant ABS control data obtained from young 'Nova' leaves sprayed with various concentrations of fluorescent pigment (0 to 2× of 1 mL L^{-1}) and copper oxychloride (0 to 2× of 2 g L^{-1}) and subsequently spray-inoculated with *Alternaria alternata*. FPC benchmarks indicating 50% and 75% control and 95% confidence intervals (CI) for means and observations are shown.

CHAPTER 3

Evaluation of two organosilicone adjuvants at reduced foliar spray volumes in South African citrus orchards of different canopy densities

This chapter has been published as: van Zyl, J.G., Sieverding, E.G., Viljoen, D.J and Fourie, P.H. 2014. Evaluation of two organosilicone adjuvants at reduced foliar spray volumes in South African citrus orchards of different canopy densities. Crop Protection.64, 198-206.

ABSTRACT

Citrus producers in South Africa generally use high spray volumes (6,000 to 16,000 L ha^{-1}) to control pests and diseases adequately for the fresh fruit market. In order to study the benefit of organosilicone adjuvants at reduced spray volumes, trials were conducted with two organo-tri-siloxane adjuvants. Two separate spray trials were conducted in the Western and Eastern Cape provinces of South Africa in uniform navel orange orchards. Break-Thru S240 (super-spreader) and Break-Thru Union (spreader-sticker), at recommended dosages per hectare (300 mL ha^{-1}, respectively), were sprayed separately in combination with a yellow fluorescent pigment (1 mL L^{-1}) at a high (20 L $tree^{-1}$ ≈ 9600 to 12 100 L ha^{-1}, depending on tree and inter-row spacing), medium (14 L $tree^{-1}$ ≈ 6500 to 8500 L ha^{-1}) and low (8 L $tree^{-1}$ ≈ 3700 to 4800 L ha^{-1}) spray application volumes. Sprays consisting of the fluorescent pigment in water alone were used as control treatments. Trees were sprayed from both sides with a commercial multi-fan tower sprayer (BSF-Multiwing) at a constant tractor speed (2.4 km h^{-1}) and spray pressure (1500 kPa). The different spray volumes were achieved by using different spray nozzles (TeeJet Disc-Core type; full and hollow cone nozzles D3-DC56/46, D4-DC56/46, D5-DC56/46). Leaves were sampled from six canopy positions (inner and outer canopy position at bottom, middle and top of the tree). Deposition quantity and quality of fluorescent pigment were determined on upper and lower leaf surfaces using fluorometry, digital photomacrography and image analyses. Spray uniformity and efficiency were also compared among treatments. Deposition quantity generally increased with increasing spray volume, but normalised values showed better spray efficiency at lower volumes. In pruned and less dense canopies, a beneficial effect of adjuvants was observed in terms of deposition quantity, efficiency and uniformity, especially at reduced volume applications (14 L $tree^{-1}$) on the inside and outside of the canopy. Little improvement in deposition quality was generally observed with the use of adjuvants. These benefits were not as evident in very dense canopies, illustrating the importance of canopy management when spraying at reduced volumes. Data obtained from the study is valuable for future improvement in spray application methodology in South Africa and other developing countries.

INTRODUCTION

South African citrus fruit producers rely heavily on medium to high volume fungicide spray applications (Grout, 1997, 2003) to protect citrus fruit from challenging diseases such as Citrus black spot (CBS) (*Phyllosticta citricarpa* (McAlpine) van der Aa (syn. *Guignardia citricarpa* Kiely)) (Kotzé, 1981, 2000; Schutte, 1997) and Alternaria brown spot (*Alternaria alternata* (Fr: Fr) Keissl., tangerine pathotype) (Schutte, 1996).

Citrus trees in South Africa are generally large and dense, with size depending on cultivar, rootstock, planting density and climatic region. Tree geometry and density complicates adequate deposition of fungicide or insecticide sprays on outer and the difficult-to-reach inner canopy susceptible leaves and fruit (Hoffmann and Salyani, 1996; Cunningham and Harden, 1998, 1999; Farooq and Salyani, 2002, 2004). Effective deposition of the active ingredient on target surfaces (leaves, twigs and fruit) is needed for effective disease control since disease control and spray deposition are closely related (Holownicki *et al*., 2002; van Zyl *et al*., 2013). Hence medium to high volume fungicide spray applications ranging from 6000 to 16 000 L ha^{-1} (with 8 000 L ha^{-1} being the norm) (Grout, 1997, 2003), which is almost double or triple the volumes used in other citrus producing counties such as Spain (Vicent *et al*., 2009) and Florida in the United States of America (Dewdney and Timmer, 2012). These application volumes do provide an acceptable balance between efficacy and efficiency based on existing economic considerations, especially considering the emphasis placed on effective CBS control given its quarantine status in certain export markets (EPPO, 2014). Most importantly, it serves as a "buffer" for loss of efficacy due to calibration and operator error and the use of inadequate spray machinery, equipment and technique. However, these high spray volumes are super-optimal, costly and not efficient in terms of time and input costs. Deposition is also not optimally efficient due to spray run-off and exo- and endo-drift (Salyani and Farooq, 2004; Fourie *et al*., 2009; Cunha *et al*., 2012; Schutte *et al*., 2012). Off-target deposition of fungicides is increased at these excessively high spray volumes (8000 L ha^{-1} and higher) (van Zyl and Fourie, unpublished results), which in turn is an economical loss and an environmental pollution problem (Salyani, 1994; Stover *et al*., 2002; Salyani and Farooq, 2004; Furness *et al*., 2006a, 2006b; de Jong *et al*., 2008; Cunha *et al*., 2012). Reduced volume sprays have the potential to reduce the economic and environmental impact/cost of fungal disease control and to be more effective (Cunningham and Harden, 1998, 1999).

Adjuvants can be used to potentially reduce spray volumes and as a result reduce application time and input costs and improve deposition parameters and disease control (Butler Ellis *et al*., 1999; Gent *et al*., 2003; Gaskin *et al*., 2004; Green and Beestman, 2007; van Zyl *et al*., 2010a, 2010b). Organomodified trisiloxanes or organosilicones as tank mix adjuvants are non-ionic surfactants that dramatically reduce surface tension and/or modify surface characteristics of hydrophobic leaves and/or fruit thereby improving wetting, spreading

and dispersing effect of the sprayed mixture on the surface or interface (Hazen, 2000). The interaction between adjuvant concentration and spray volume and to some extent the effect it has on the biological efficacy of certain crop protection products (Greyson *et al*., 1995, 1996) has been studied on fruit (Stevens *et al*., 1994, 1995), easy to wet foliage of potatoes (Greyson *et al*., 2006) and on difficult to wet foliage of wheat (Greyson *et al*., 1996; Gaskin and Murray, 1997). In all cases, depending on the surface characteristics of the sprayed target, it was found that increased concentration of adjuvant or organosilicone at increased spray volumes led to increased spray run-off and reduced retention, whereas, increased concentration of organosilicone and decreased spray volume had the opposite effect (de Ruiter *et al*., 1990; Stevens *et al*., 1993; Gaskin and Murray, 1997; Gaskin *et al*., 2000). This interaction and its results are likely to influence the biological efficacy of crop protection products deposited.

In South Africa, these types of adjuvants are regularly used with fungicide and pesticide application, yet little literature exists on the effect of adjuvants, specifically organo tri-siloxane adjuvants at different application volumes in citrus canopies. Therefore, the objectives of this study were to evaluate the influence of two organo tri-siloxane surfactants at reduced application volumes in South African citrus orchards on different deposition parameters. In previous studies, an spray deposition assessment protocol, consisting of fluorometry, digital photomacrography and image analysis, was developed and improved (Brink *et al*., 2004, 2006; Fourie *et al*., 2009; van Zyl *et al*., 2010a, 2010b; van Zyl *et al*., 2013) and was recently used to effectively determine the deposition quantity (the amount of active ingredient landed and retained on a target surface) and persistence (amount of product retained over time) of copper fungicides on orange leaves and fruit (Schutte *et al*., 2012). Recent improvements to the spray deposition assessment protocol also allows for determining deposition quality (uniformity of active ingredient distribution on the target surface) (J.G van Zyl; unpublished results). This deposition assessment protocol was used in this study.

MATERIALS AND METHODS

Spray application

Field evaluation

The first trial (Dense canopy trial) was conducted in uniform (areas where trees have similar canopy characteristics e.g. height, width and density) sections of a 14-year-old ‘Bahianina Araras’ navel (*Citrus sinensis* (L.) Osbeck) orchard on the farm Die Vlei (Clanwilliam, Western Cape, South Africa) in February 2012. Trees were large [3.7 × 3.4 m trees H × W (height × width or depth across row)] and planted at a 3 × 5.5 m tree and inter-row spacing. Canopy density was visually determined on a 5-point scale (1 - very sparse leaf concentration, heavily aerated; 2 - sparse leaf volume, well aerated; 3 - good balance between leaf volume and canopy aeration; 4 - dense canopy, sparsely aerated; 5 - very dense leaf concentration, poorly

aerated with no pruned canopy windows, *i.e.* unmanaged) and was rated to have a density of 4.5. Sprays were applied early morning as soon as trees were dry from dew. Air movement (wind speed at m s^{-1}) inside the orchard row was 1.8 m s^{-1} with air temperature and relative humidity being 26ºC and 18%, respectively. For each treatment combination, a single row-section of 10 trees was marked and sprayed from both sides with a tractor-drawn, power take-off (PTO) powered, air-blast BSF-Multiwing sprayer (BSF, Hoedspruit, South Africa). The spray applicator is a high-profile sprayer with 22 nozzle ports per side, with air being generated by five fans per side positioned vertically behind the nozzles without ducting on the sprayer tower, to match canopy height. A high-profile sprayer was used to negate the effects of using low profile applicators in large canopies (Cunningham and Harden, 1998; van Zyl and Fourie unpublished results). Spray volume was standardised to L $tree^{-1}$ since inter-row spacing differed between the two trials sprayed. Even though spray volume differed between trials, the volume per tree was similar. Sprays consisted of three separate treatments, each at a high (20 L $tree^{-1}$ = 12 273 L ha^{-1}), medium (14 L $tree^{-1}$ = 8273 L ha^{-1}) and low (8 L $tree^{-1}$ = 4727 L ha^{-1}) spray volume. The three separate treatments contained a yellow fluorescent pigment [40% EC (SARDI, Loxton, South Australia) at 1 mL L^{-1}] alone (no adjuvant added control treatment), yellow fluorescent pigment (1 mL L^{-1}) together with adjuvant Break-Thru S240 [(Evonik Degussa Africa, Midrand, South Africa) at 300 mL ha^{-1}] and yellow fluorescent pigment (1 mL L^{-1}) together with Break-Thru Union [(Evonik Degussa Africa, Midrand, South Africa) at 300 mL ha^{-1}]. Break-Thru S240 is a super-spreader, trisiloxane-based adjuvant that enables extremely low surface tension of aqueous solutions whilst causing super-spreading of droplets due to droplet size diameter increase. Break-Thru UNION is a spreader-sticker trisiloxane-based adjuvant that increases wetting and adhesion on target surfaces and reduces drift potential due to large increase in droplet size. Tractor speed, PTO speed and spray pressure were kept constant at 2.4 km h^{-1}, 540 rpm and 1500 kPa, respectively, with spray volume being manipulated by using different spray nozzles (TeeJet Disc-Core type full and hollow cone nozzles: Low - D3-DC56/46, medium - D4-DC56/46, High - D5-DC56/46). Two buffer rows of trees were left unsprayed between treatment blocks. The spray tank, spray nozzles, filter and pipes of the spray machine were thoroughly washed and flushed after each treatment.

The second trial (Open canopy trial) was sprayed in uniform sections of a well-pruned 'Palmer' navel (*Citrus sinensis* (L.) Osbeck) [3.65×3.2 m trees (H×W) orchard with a canopy density of 2.5 on a 5-point scale; 3×7 m tree and inter-row spacing] orchard on the farm Sun Orange Farms (Addo, Eastern Cape, South Africa) in April 2012. Spray application was conducted early morning with in-row air movement measured at 2 m s^{-1}. Temperature during application was 22ºC and relative humidity 34%. Apart from the tree canopies being well aerated (less dense), the methodology used was exactly the same as the first trial. Spray

volumes per tree was the same as the first trial at high (20 L tree^{-1}), medium (14 L tree^{-1}) and low (8 L tree^{-1}) volume applications, but realised lower per hectare rates (9643 L ha^{-1}, 6500 L ha^{-1} and 3714 L ha^{-1}, respectively) at the wider inter-row spacing (7 m).

Sampling of field evaluations

As replications, three random uniform trees were selected from each sprayed section (treatment) from which leaves were sampled for spray deposition analysis. After the spray mixture had dried, twelve randomly selected intact leaves were carefully sampled from each of the various positions in the tree canopy; inner (>30 to 50 cm into the tree) and outer canopy (leaves on the outside of the tree) at the top, middle and bottom parts of each of the selected trees (72 leaves per replication). Leaves picked from these six positions were collected and stored separately in marked polyethylene sandwich bags. Stored leaves were transported back under cool, dry conditions to the laboratory where they were stored at 4°C until further analysis.

Spray deposition analysis

For deposition analysis, petioles were removed from leaves using a pair of scissors at the base of the leaf blade. A single leaf was positioned in the middle of a back-illuminated red Perspex box (300×210×110 mm) inside a dark room to reduce any shadowing and to enhance edging of leaves in captured images during analysis. The leaf was covered with a glass pane (200×200×2 mm) and illuminated using an ultra-violet light source (UV-A; ≈ 365 nm; Labino Mid-Light; www.labino.com). Digital photos were taken in Canon RAW file format (.CR2 ≈ 10 MB) of the adaxial and abaxial leaf surfaces using a Canon EOS 40D camera equipped with a 60 mm macro lens. The camera was attached to a tripod in a fixed position directly above the leaf. RAW image files were converted to 8-bit Exif-TIFF (.TIF ≈ 30 MB) with Digital Photo Professional version 3.1.0.0 (CANON INC.; www.canon.com) files for digital image analysis to determine the deposition parameters (van Zyl *et al*., 2013).

Spray deposition assessment involved digital image analysis with Image Pro Plus software version 7.0 (Media Cybernetics, www.mediacy.com) to determine the deposition quantity and quality per leaf. Similar to the methodology used in van Zyl *et al*. (2013), deposition quantity was measured as percent total leaf area covered by pigment particles (percentage fluorescent particle coverage; FPC%) (van Zyl *et al.*, 2013). For the deposition quality assessment, the leaf area was divided into equally-sized squares [100 × 100 pixels (10000 pixels)]. Depending on the leaf size, this amounted to as few as 20 to more than 250 individual squares per leaf, of which the percent area covered by fluorescent pigment particle was determined for each square. The Interquartile Coefficient of Dispersion (ICD%), a form of the Coefficient of Quartile Variation (CQV) (Bonnet, 2006), per leaf [((3rd quartile – 1st

quartile)/(3rd quartile + 1st quartile))*100] was used as a measure of deposition quality per leaf, *i.e.* uniformity of deposition on the leaf surface. Low interquartile coefficient of dispersion values was indicative of better deposition quality. This analysis method is an improvement on previously used methodology (van Zyl *et al*., 2013). Deposition uniformity between leaves was calculated as the CV% in pigment deposition in a 12-leaf batch (Standard Deviation × 100/mean) and deposition efficiency was expressed as deposition quantity normalised to FPC% per L tree^{-1}.

Benchmarking

Deposition data were subjected to the FPC benchmark model developed by van Zyl *et al*. (2013) to evaluate the effectiveness of deposition in relation to theoretical disease control that can be achieved on foliage. The FPC_{50} (2.07 FPC%) and FPC_{75} (4.14 FPC%) benchmarks indicate 50% and 75% theoretical control of Alternaria Brown Spot on mandarin leaves, respectively.

Statistical analysis

A completely random split plot design with treatment as main plot factor, position within each tree canopy as subplot factor and leaf surface (upper/lower) as sub-subplot factor was used. Deposition quantity (FPC%), quality (ICD%), uniformity (CV%) and efficiency (FPC% per L tree^{-1}) data were subjected to appropriate analysis of variance (ANOVA). Fisher's LSD was calculated to identify significant differences between treatments at a confidence interval of 95%. Data from upper and lower leaf surfaces were analysed separately, but were combined when describing the results. Data was also subjected to regression analysis and Pearson's correlation to demonstrate the possible relations between deposition quantity, quality and uniformity measurements. SAS version 8.2 statistical software (SAS institute Inc., 1999) was used for analysis.

RESULTS

Analysis of variance indicated significant interactions between trials. These differences between trials were largely ascribed to canopy density and the trials were therefore analysed separately. Unless significant higher order interactions were observed from the analysis of variance, the 2-factor treatment × volume and treatment × horizontal canopy position interactions were discussed to simplify interpretation of subtle treatment effects.

Dense canopy trial – 'Bahianina Araras' navel orchard (Clanwilliam, Western Cape)

Deposition quantity

Analysis of variance of deposition quantity (FPC%) indicated no significant interactions ($P < 0.05$), but some significant main effects: vertical canopy position ($P < 0.0001$), horizontal canopy position ($P = 0.0015$) and spray volume ($P = 0.0078$). A somewhat lower, but yet arguably meaningful effect was also observed for treatment effects ($P = 0.0756$).

For vertical canopy position, the highest deposition was realised at the top of the tree (6.2 FPC%) which differed significantly from that at the bottom (4.79 FPC%) and the middle of the tree (4.57 FPC%), which did not differ significantly from each other.

Evaluation of the treatment × volume interaction ($P = 0.531$), indicated that the control treatment of water only (7.28 FPC%) and the Break-Thru S240 treatment (7.49 FPC%) retained the highest amount of pigment on leaves at 20 L tree^{-1}, statistically more than that retained by all other treatments. Pigment retained by Break-Thru Union at 20 L tree^{-1} (5.85 FPC%) and 14 L tree^{-1} (5.05 FPC%) was statistically lower than that of above-mentioned treatments and did not differ significantly from the amount retained on leaves by water only at 14 L tree^{-1} (5.82 FPC%). The lowest amount of pigment was retained by Break-Thru Union at 8 L tree^{-1} (3.46 FPC%) (Table 1).

For horizontal canopy position, treatments generally deposited higher deposition quantities on the outside of the tree (5.65 FPC%) in relation to the inside of the canopy (4.72 FPC%) over all treatments. There was no significant interaction for treatment × horizontal canopy position ($P = 0.532$). For water and Break-Thru S240, deposition quantities on outer and inner canopy leaves were not significantly different, but inner canopy deposition was significantly lower for Break-Thru Union (Table 2).

Deposition uniformity

Analysis of variance of deposition uniformity between leaves (in a 12-leaf batch) indicated a significant interaction for treatment × vertical canopy position ($P = 0.0251$) and a significant effect for horizontal canopy position ($P = 0.0501$).

The treatment × volume interaction, although not significant ($P = 0.676$), was discussed for better interpretation of the data. Break-Thru Union at 8 L tree^{-1} improved deposition uniformity (58.47 CV%) in relation to water only (68.07 CV%) and S240 (67.54 CV%) treatments. Although not statistically significant, this marginal effect might prove meaningful under field conditions and should be researched further. This marginal effect was also observed at 20 L tree^{-1}, but not at 14 L tree^{-1} (Table 1).

For the significant interaction treatment × vertical canopy position, the least variation in deposition uniformity was realised by water only treatment (52.63 CV%) in the bottom of the canopy whilst the highest variation in deposition between leaves were realised by water only

in the top of the canopy (67.39 CV%), differing statistically from that of the lowest variation. Deposition uniformity realised by adjuvant treatments did not improve at different canopy positions in relation to the control treatment (results not shown).

Adjuvant treatments did not improve deposition uniformity significantly on the inside or outside of the canopy in relation to the control treatment (57.93 and 62.39 CV%, respectively) (Table 1).

Deposition efficiency

Analysis of variance of deposition efficiency [normalised deposition FPC% to a spray volume of 1 L tree^{-1})] indicated no significant interactions ($P > 0.05$), but some significant main effects: vertical canopy position ($P < 0.0001$) and for horizontal canopy position ($P < 0.0001$). A meaningful effect was also observed for treatment effects ($P = 0.0582$).

The interaction treatment × volume ($P = 0.237$) and treatment × horizontal canopy position ($P = 0.267$) was evaluated for deposition efficiency. At 8 L tree^{-1}, the water only treatment (0.51 FPC% per L tree^{-1}) realised the best deposition efficiency, and Break-Thru S240 (0.45 FPC% per L tree^{-1}) and Union (0.43 FPC% per L tree^{-1}) realised similar deposition efficiency at the same volume. At 14 L tree^{-1}, Break-Thru S240 (0.37 FPC% per L tree^{-1}) and water only (0.36 FPC% per L tree^{-1}) realised the best deposition efficiency, but not differing statistically from the markedly lower deposition efficiency realised by Break-Thru Union (0.29 FPC% per L tree^{-1}). At 20 L tree^{-1}, deposition efficiency following water only (0.42 FPC% per L tree^{-1}) sprays was significantly better than that of Break-Thru S240 (0.29 FPC% per L tree^{-1}), but similar to Break-Thru Union (0.36 FPC% per L tree^{-1}) (Table 1). On outer canopy leaves, adjuvant treatments realised less deposition efficiency, although not significantly less than the water only control treatment on the outer canopy. However, on inner canopy leaves, water only (0.40 FPC% per L tree^{-1}) realised statistically better deposition efficiency than that of the adjuvant treatments (S240 – 0.33 FPC% per L tree^{-1} and Union – 0.29 FPC% per L tree^{-1}) (Table 2).

Deposition quality

Analysis of variance of deposition quality (ICD%) indicated significant interactions for treatment × volume ($P = 0.0331$) and a meaningful interaction for treatment × horizontal canopy position ($P = 0.0633$). The least variation in pigment distribution was realised by the water-only treatment applied at 8 L tree^{-1} (51.58 ICD%), significantly better than S240 (56.4 ICD%) and markedly better than Union (54.12 ICD%) at this spray volume. This was also the case at 14 L tree^{-1} (52.32 ICD%); significantly better than Union (59.16 ICD%) and marginally better than S240 (55.82 ICD%). Statistically similar deposition quality levels (52.45 – 56.37

ICD%) were realised between treatments at 20 L tree^{-1}, with Break-Thru S240 (52.45 ICD%) realising the lowest variation in pigment distribution (Table 1).

Evaluating the interaction treatment × horizontal canopy position, overall better deposition quality was realised by Break-Thru S240 and Union on inner canopy leaves (52.42 and 55.63 ICD%, respectively), marginally better than on outer canopy leaves (57.37 and 57.4 ICD%, respectively). However, sprays with water only realised better deposition quality on the inner (52.65 ICD%) and outer canopy leaves (53.31 ICD%) (Table 2).

Benchmarking

Deposition quantity results for the treatment × volume × horizontal canopy position interaction were compared to the FPC benchmarks (van Zyl *et al*., 2013). All treatments at 8, 14 and 20 L tree^{-1} obtained deposition quantity levels sufficiently above the FPC_{50} and FPC_{75} benchmarks on the outside of the canopy, indicating sufficient deposition quantity for control above 75%. However, on the inside of the canopy, all treatments at 8 L tree^{-1} realised deposition quantities above the FPC_{50} but below the FPC_{75} benchmarks. At 14 L tree^{-1}, the water only treatment and Break-Thru Union deposited quantities above the FPC_{75} benchmark. At 20 L tree^{-1}, deposition realised by the control treatment and adjuvants were sufficiently above the FPC_{75} benchmark (Figure 1).

Open canopy trial – 'Palmer' navel orchard (Addo, Eastern Cape)

Deposition quantity

Analysis of variance of deposition quantity (FPC%) indicated a significant interaction for treatment × volume (P = 0.0047) and no significant interaction for treatment × horizontal canopy position (P = 0.218).

At 8 L tree^{-1}, Break-Thru Union (4.66 FPC%) realised markedly, but not significantly, higher deposition quantity than the water only (3.59 FPC%) and Break-Thru S240 (3.73 FPC%) treatments. At 14 L tree^{-1}, the adjuvants resulted in similar deposition quantities (5.84-5.85 FPC%), significantly higher than the water only control treatment (3.42 FPC%). At 20 L tree^{-1}, Break-Thru Union (7.17 FPC%) realised significantly higher deposition quantity than water only (5.45 FPC%) and Break-Thru S240 (4.54 FPC%) (Table 3).

For the treatment × horizontal canopy position interaction, Break-Thru Union realised significantly higher deposition quantity on outer canopy leaves (6.39 FPC%) than Break-Thru S240 (4.78 FPC%) and water only (4.63 %). On inner canopy leaves, adjuvant treatments Break-Thru Union (5.40 FPC%) and S240 (4.54 FPC%) realised significantly higher deposition quantities than water only treatment; Union significantly better than S240 (Table 4).

Deposition uniformity

Analysis of variance of deposition uniformity between leaves (in a 12-leaf batch) indicated a significant interaction for treatment × volume (P = 0.0359) and no significant interaction for treatment × horizontal canopy position (P = 0.217).

At 8 L tree^{-1}, Break-Thru Union realised significantly better deposition uniformity (61.31%) than the control treatment (72.80 CV%), but similar to S240 (65.18 CV%). At 14 L tree^{-1}, Break-Thru S240 (54.22 CV%) realised the best deposition uniformity, significantly better than that of the control treatment (71.42 CV%) and Break-Thru Union (64.13 CV%). Deposition uniformity realised by adjuvant treatments did not differ significantly from that of the control treatment at 20 L tree^{-1} (54.91-64.13 CV%; Table 3).

For the treatment × horizontal canopy position interaction, Break-Thru S240 and Union improved deposition uniformity on outer canopy leaves (61.66 and 55.85 CV%, respectively) in relation to the water only treatment (66.07 CV%), with Union improving uniformity significantly. On inner canopy leaves, both adjuvants (60.69 and 64.38 CV%, respectively) improved deposition uniformity over that of the control treatment (70.35 CV%), with Break-Thru S240 doing so significantly (Table 4).

Deposition efficiency

Analysis of variance of deposition efficiency indicated significant interactions for treatment × horizontal canopy position × vertical canopy position (P = 0.0281) and for treatment × volume (P = 0.0147), but not for treatment × horizontal canopy position (P = 0.240).

For the treatment × volume interaction, Break-Thru Union at 8 L tree^{-1} (0.58 FPC% per L tree^{-1}) improved deposition efficiency significantly in relation to the deposition efficiency achieved by the control treatment (0.47 FPC% per L tree^{-1}) and Break-Thru S240 (0.45 FPC% per L tree^{-1}). At 14 L tree^{-1}, adjuvant treatments did not differ from each other (0.42 FPC% per L tree^{-1}) and significantly improved deposition efficiency compared to the control treatment (0.24 FPC% per L tree^{-1}). At 20 L tree^{-1}, Break-Thru Union again realised the best deposition efficiency; significantly better than the control treatment (0.27 FPC% per L tree^{-1}) and S240 (0.22 FPC% per L tree^{-1}) (Table 3).

Break-Thru Union realised significantly better deposition efficiency on outer canopy leaves (0.49 FPC% per L tree^{-1}) than the control treatment (0.36 FPC% per L tree^{-1}) and Break-Thru S240 (0.38 FPC% per L tree^{-1}). On inner canopy leaves, both Break-Thru Union (0.41 FPC% per L tree^{-1}) and S240 (0.35 FPC% per L tree^{-1}) improved deposition efficiency significantly compared to the control treatment water only (0.29 FPC% per L tree^{-1}) (Table 3).

For the interaction treatment × horizontal canopy position × vertical canopy position, best deposition efficiency was realised by Break-Thru Union (0.54 FPC% per L tree^{-1}) on outer canopy leaves in the top of trees; significantly better than what was realised by the control

treatment (0.35 FPC% per L tree^{-1}) and Break-Thru S240 (0.40 FPC% per L tree^{-1}) at this position. On outer canopy leaves in the middle position on trees, Break-Thru Union and the water only control treatment realised similar deposition efficiency (0.44 FPC% per L tree^{-1}), significantly better than Break-Thru S240 (0.40 FPC% per L tree^{-1}). On outer canopy leaves in the bottom of trees, Break-Thru Union and S240 realised the best deposition efficiency (0.39 to 0.50 FPC% per L tree^{-1}), significantly better than that of the control treatment (0.30 FPC% per L tree^{-1}). On inner canopy leaves in tops of trees, Break-Thru Union (0.38 FPC% per L tree^{-1}) realised the best deposition efficiency, significantly better than the control treatment (0.23 FPC% per L tree^{-1}), whilst Break-Thru S240 realised an intermediate efficiency level (0.30 FPC% per L tree^{-1}) that did not differ significantly from the control treatment and Union. Similar trends were observed in the middle and bottom of trees on inner canopy leaves, where Break-Thru Union realised the best deposition efficiency, significantly better than the control treatment, and with Break-Thru S240 being intermediate between the control treatment and Union (results not shown).

Deposition quality

Analysis of variance of deposition quality (ICD%) indicated a significant interaction for treatment × horizontal canopy position (P = 0.0435), but not for treatment × volume (*P* = 0.4434), no significant differences were observed between treatments (55.41 -59.12 ICD%), except between the best and worst treatments, water only at 8 L tree^{-1} and Union at 14 L tree^{-1}, respectively (Table 3).

Deposition quality levels on inner canopy leaves was lower (better) than on outer canopy leaves, with deposition on inner canopy leaves being similar for adjuvants and the water only treatment (53.33 to 54.80 ICD%). On outer canopy leaves, Break-Thru Union realised the lowest deposition quality (61.77 ICD%) significantly lower than that realised by Break-Thru S240 (58.79 ICD%). The water only treatment on outer canopy leaves was intermediate (60.98 ICD%) (Table 4).

Benchmarking

Deposition quantity results for the treatment × volume × horizontal canopy position interaction were compared to the FPC benchmarks (van Zyl *et al*., 2013). On outer canopy leaves, all treatments realised deposition quantities above the FPC_{75} benchmark, except for Break-Thru S240 at 8 L tree^{-1} and the control treatment at 14 L tree^{-1}, only realising deposition quantities above the FPC50 benchmark. On inner canopy leaves, Break-Thru Union realised deposition quantities above the FPC_{75} benchmark at all spray volumes. At 8 L tree^{-1}, the control treatment and Break-Thru S240 only realised deposition quantities above the FPC_{50} benchmark. At 14 L tree^{-1}, the control treatment deposited deposition quantities above the FPC_{50} benchmark,

but below the FPC_{75} benchmark. At 20 L $tree^{-1}$, all treatments realised deposition quantities better than the FPC_{75} benchmark (Figure 2).

DISCUSSION

This study evaluated the influence of organosilicone adjuvants Break-Thru S240 and Break-Thru Union on deposition parameters at high and reduced spray volumes throughout citrus tree canopies. As in previous studies, the use of fluorometry, photomacrography, digital image analysis (Schutte *et al*., 2012; van Zyl *et al*., 2013) and implementation of deposition benchmarks (for biological interpretation of deposition results) (van Zyl *et al*., 2013) was highly effective in determining, evaluating and visualising deposition parameters of treatments. The fluorescent pigment used was proven by van Zyl *et al*. (2013) to be an accurate tracer for contact copper fungicide deposition and was therefore used in this study.

Markedly different results were obtained between the two trials. Differences were largely ascribed to the variation in canopy density between the two trials. Unfortunately canopy density was not quantified. The crude 5-point scale used was effective in discerning between different canopy densities but should be improved in future studies. Differences between treatments were ascribed to the effects of adjuvant formulation on droplet formation (Spanoghe *et al*., 2007) and subsequently, canopy penetration (Gent *et al*., 2003) and deposition (de Ruiter *et al*., 1990; Holloway *et al*., 2000) of the fluorescent pigment on leaf surfaces. Droplet size was not determined in our study. The same spray machines were used in both trials and they were similarly calibrated whilst tractor speed and spray pressure used was to our knowledge, the most ideal for these spraying conditions. Tractor speed, PTO speed and spray pressure (2.4 km h^{-1}, 540 rpm and 1500 kPa, respectively) were kept constant throughout treatments and both trials to limit the variable effect on deposition parameters (Whitney *et al*., 1989; Salyani and Whitney, 1990). These factors are very important, since improper calibration, speed and pressure selection along with wrong spraying techniques, are usually the reason for poor deposition and therefore most treatment failures (Salyani, 1994; Grout, 1997, 2003; Stover *et al.,* 2002).

In both trials, deposition quantity generally increased with increase in spray volume for all treatments. Similar deposition was achieved throughout the top, middle and bottom of the canopy, except for better deposition achieved in the top of the canopy in trial one. This is testament to the efficacy of tower sprayers, as opposed to low-profile sprayers that generally deposited lower deposition quantities in tops of trees (P.H. Fourie, unpublished results). Deposition quantity was also found to be higher on outer canopy leaves than on inner canopy leaves with deposition at these positions increasing with spray volume. Our findings on spray deposition in citrus orchards support those of Farooq and Salyani (2002) previous studies.

However, we found an increase in deposition on inner canopy leaves with an increase in spray volume, contradictory to that found by Salyani *et al*. (1988).

Canopy density had a direct effect on deposition quantity on outer and inner canopy leaves, with deposition quantity realised on outer and inner canopy leaves being higher and more consistent per treatment than that realised on dense canopies. This concurs with Salyani and Whitney (1990) and Farooq and Salyani (2002) who found higher variation in deposition at different positions in dense canopies. It has to be stated that different deposition measurement protocols were used in the studies mentioned but still similar outcomes were found.

The addition of Break-Thru S240 and Union to sprays at 8- 14- and 20 L tree^{-1}, did not improve deposition quantity or uniformity throughout the canopy (inner and outer canopy leaves) in dense canopies (trial one). In fact, a detrimental effect in terms of deposition quantity and uniformity was observed, especially with Break-Thru Union at 20 L tree^{-1} and on inner canopy leaves. However, in less dense, pruned canopies, the addition of adjuvants to sprays had a beneficial effect with deposition quantity and uniformity realised being higher than that realised by the water only sprays. Improved deposition quantity on inner canopy leaves was especially evident with Break-Thru S240 and Break-Thru Union at 14 L tree^{-1}. A possible explanation for this phenomenon could be that droplets from sprays with Break-Thru S240 and Union impacting the dense canopy wall formed a film due to reduced surface tension and better adhesion of the spray mixture, which possibly led to increased run-off and reduced canopy penetration at the higher spray volumes. With the water only spray at these spray volumes, impacting water droplets on the canopy wall might have physically shattered on the hydrophobic leaf surfaces, since little to no film forming took place. Smaller shattered droplets could possibly drift more easily through the canopy wall onto inner canopy leaves. In less dense, pruned canopies, more uniform adjuvant droplets could be carried through the outer canopy since it was not captured by the dense outer canopy.

Deposition quantity following sprays at 20 L tree^{-1} was judged as sufficient in both trials as levels were above the FPC_{75} benchmark in all cases (van Zyl *et al*., 2013; Figure 1 and 2). However, when considering spray efficiency as well as benchmarks, spray deposition following sprays with S240 and Union at 14 L tree^{-1} in less dense, pruned canopies (trial 2) was most effective. The improvement of deposition uniformity in less dense compared with dense canopies was also evident. Deposition uniformity was improved with the addition of the two adjuvants at different spray volumes on the outside of the canopy, and most importantly on the inner canopy leaves in pruned canopies. In less dense canopies, this was only the case for Break-Thru Union, which improved deposition uniformity at all spray volumes and also on the inner and outer canopy.

This study highlights the importance of canopy management. Canopy density was an important factor. More aerated, pruned canopies were essential for improved deposition, since spray mixture could readily deposit and penetrate the canopy due to improved air-movement. In more dense canopies, penetration of the "leaf wall" at all volumes was more difficult, causing excessive run-off on the outer canopy before spray mixture could readily deposit and penetrate the canopy. Run-off from outer canopy leaves could have been exacerbated by the addition of an adjuvant in these dense canopies, due to the reduction in surface tension, causing a run-off "flushing" effect as droplets impacted on leaf surfaces, not deflecting or fracturing off the leaf surface, creating smaller fractured droplets that could more readily have been carried to the inside of the canopy. This phenomenon could theoretically be the reason for relatively poor deposition on the inner canopy leaves following adjuvant sprays in dense canopies (trial 1).

In terms of deposition efficiency, Break-Thru Union proved to be superior at all spray volumes in less dense canopies, especially at lower volume applications (8 L tree^{-1}), as also observed with deposition quantity. In dense canopies, the addition of the adjuvants did not improve deposition efficiency.

From the results obtained it is clear that canopy management is of cardinal importance for improving spray deposition, especially for reduced volume applications. If a canopy is not well managed and pruned (*i.e.* "spray friendly"), for example, does not have pruned windows to the inside of the canopy and is too dense, spray deposition will be negatively affected and will result in loss of effectiveness of spray application and through it, reduced disease control. Furthermore, the benefits of an adjuvant were especially evident in less dense "spray-friendly" canopies. A definite beneficial was observed with the adjuvants, especially at lower spray volume applications (8 and 14 L tree^{-1}), indicating the potential to improve deposition quantity, efficiency and uniformity on the inner and outer canopy leaves, provided that the canopy is less dense, pruned.

ACKNOWLEDGEMENTS

The authors acknowledge Citrus Research International and Evonik South Africa for funding; Farmers for the trial sites and spray machines and Marieta Van Der Rijst (ARC-Biomet, Stellenbosch) for statistical analysis.

REFERENCES

Bonnet, D.G., 2006. Confidence interval for a coefficient of quartile variation. Computational Statistics and Data Analysis 50: 2953-2957.

Brink, J.C., Calitz, F.J., Fourie, P.H., Holz, G., 2004. Development of a protocol to quantifiy spray deposits on grape bunches. In: 7th International Symposium on Adjuvants for Agrochemicals ISAA 2004. ISAA 2004 Foundation, South Africa, pp. 230-236.

Brink, J.C., Fourie, P.H., Holz, G., 2006. Effect of fungicide spray cover on Botrytis cinerea infection in grape bunches. South African Journal for Enology and Viticulture 27: 51-56.

Butler Ellis, M.C. and Tuck, C.R. 1999. How adjuvant influence spray formation with different hydraulic nozzles. Crop Protection 18: 101-109.

Cunha, J.P., Chueca, P., Garcerá, C., Moltó, E. 2012. Risk assessment of pesticide spray drift from citrus applications with air-blast sprayers in Spain. Crop Protection 42: 116-123.

Cunningham, G.P., Harden, J., 1998. Air-tower sprayers increase application efficiency in mature citrus trees. Australian Journal of Experimental Agriculture 38: 871-877.

Cunningham, G.P., Harden, J., 1999. Sprayers to reduce spray volumes in mature citrus trees. Crop Protection 18: 275-281.

De Jong, F.M.W., de Snoo, G.R., van de Zande, J.C., 2008. Estimated nationwide effects of pesticide spray drift on terrestrial habitats in the Netherlands. Journal of Environmental Management 86: 721-730.

De Ruiter, H., Uffing, A.J.M., Meinen, E., Prins, A., 1990. Influence of surfactants and plant species on leaf retention of spray solutions. Weed Science 38: 567-572.

Dewdney, M.M., Timmer, L.W., 2012., 2012 Florida citrus pest management guide: Alternaria brown spot. PP-147, series of the Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu.

EPPO,. 2014. 2014 PQR - EPPO database on quarantine pests (available online). http://www.eppo.int.

Farooq, M., Salyani, M., 2002. Spray penetration into the citrus tree canopy from two air-carrier sprayers. Transactions of the American Society of Agricultural Engineers 45: 1287-1293.

Farooq, M., Salyani, M., 2004. Modelling of spray penetration and deposition on citrus tree canopies. Transactions of the ASABE 47: 619-627.

Furness, G.O., Manktelow, D.W.L., Thompson, A.J., 2006a. A Visual droplet number rating chart and fluorescent pigment sprays to estimate chemical deposition and spray coverage on plant foliage. Aspects of Applied Biology 77: 171-178.

Furness, G.O., Manktelow, D.W.L., Thompson, A.J., 2006b. Multi-fan spray towers to improve dose efficiency and spray coverage uniformity in citrus trees. Aspects of Applied Biology 77: 481-488.

Fourie, P.H., du Preez., M., Brink, J.C., Schutte, G.C., 2009. The effect of runoff on spray deposition and control of Alternaria brown spot of mandarins. Australasian Plant Pathology 38: 173-182.

Gaskin, R.E., Murray, R.J., 1997. Effect of surfactant concentration and spray volume on retention of organosilicone sprays on wheat. Proceedings of the 50th New Zealand Plant Protection Conference 1997: 139-142.

Gaskin, R.E., Elliott, G.S., Manktelow, D.W.L., Skinner, S.J., 2004. Use of a superspreader adjuvant to reduce spray application volumes on avocados. New Zealand Plant Protection 57: 266-270.

Gent, D.H., Schwartz, H.F., Nissen, S.J., 2003. Effect of commercial adjuvants on vegetable crop fungicide coverage, absorbtion and efficacy. Plant Disease 87: 591-597.

Green, J.M., Beestman, G.B., 2007. Recently patented and commercialized formulation and adjuvant technology. Crop Protection 26: 320-327.

Greyson, B.T., Boyd, S.L., Sampson, A.J., Drummond, J.N., Walter, D. 1995. The effect of adjuvants on the performance of the new cereal fungicide, metconazole. I: Glasshouse trials. Pesticide Science 45: 153-60.

Greyson, B.T., Price, P.J., Walter, D. 1996. Effect of the volume rate of application on the glasshouse performance of crop protection agent/adjuvant combinations. Pesticide Science 48: 205-217.

Grout, T.G., 1997. Spray volumes and coverage requirements for citrus in South African Citrus Journal 31: 19-20.

Grout, T.G., 2003. Use of plant protection products. Section 2.7 in: Grout, T.G. (Ed.), Citrus production guidelines. Integrated Pest Management. Vol III. Citrus Research International, Nelspruit, South Africa.

Hazen, J. L., 2000. Adjuvants-Terminology, classification, and chemistry. Weed Technology 14: 773-784.

Hoffmann, W.C., Salyani, M. 1996. Spray deposition on citrus canopies under different meteorological conditions. Transactions of the American Society of Agricultural Engineers 39: 17-22.

Holloway, P.J., Butler Ellis, M.C., Webb, D.A., Western, N.M., Tuck, C.R., Hayes, A.L., Miller, P.C.H., 2000. Effects of some agricultural tank-mix adjuvants on deposition efficiency of aqueous sprays on foliage. Crop Protection 19: 27-37.

Holownicki, R., Doruchowski, G., Jaeken, P., Swiechowski, W., 2002. Methods of evaluation of spray deposit and coverage on artificial targets. Electronic Journal of Polish Agricultural Universities (EJPAY) 5 (1), #3. Online publication.

Kotzé, J.M., 1981. Epidemiology and control of citrus black spot in South Africa. Plant Disease 65: 945-950.

Kotzé, J.M., 2000. Citrus black spot. Pages 23-25 in: Compendium of Citrus Diseases (second Edition). Timmer, L.W., Garnsey, S.M. and Graham, J.H. APS Press, The American Phytopathological Society, St. Paul Minnesota, USA.

Salyani, M., Farooq, M., 2004. Drift potential of citrus air-carrier sprayers. Proceedings of the Florida State Horticultural Society 117: 130-135.

Salyani, M., Whitney, J.D., 1990. Ground speed effect on spray deposition inside citrus trees. Transactions of the American Society of Agricultural Engineers 33: 361-366.

Schutte, C.G., 1996. First report of Alternaria brown spot on Star Ruby grapefruit in South African Citrus Journal 6: 24.

Schutte, G.C., Beeton, K.V., Kotzé, J.M., 1997. Rind stippling on Valencia oranges by copper fungicides used for control of citrus black spot in South Africa. Plant Disease 81: 851-854.

Schutte, G.C., Kotze, C., van Zyl, J.G., Fourie, P.H., 2012. Assessment of retention and persistence of copper fungicides on orange fruit and leaves using fluorometry and copper residue analyses. Crop Protection 42: 1-9.

Spanoghe, P., De Schampheleire, M., Van der Meeren, P., Steurbaut, W., 2007. Influence of agricultural adjuvants on droplet spectra. Pest Management Science 63: 4-16.

Stevens, P.J.G., Kimberley, M.O., Murphy, D.S., Policello, G.A., 1993. Adhesion of spray droplets of foliage: the role of dynamic surface tension and advantages of organosilicone surfactants. Pesticide Science 38: 237-245.

Stover, E., McClure, P., Salvatore, J., 2002. Influence of spray volume, ground speed, and a sensor-actuated spray controller on melanose control and fruit marking in fresh Indian river grapefruit. Proceedings of the Florida State Horticultural Society 115: 1-5.

van Zyl, J.G., Fourie, P.H., Schutte, C.G., 2013. Spray deposition assessment and benchmarks for control of Alternaria brown spot on mandarin leaves with copper oxychloride. Crop Protection 46: 80-87.

van Zyl, S.A., Brink, J.C., Calitz, F.J., Coertze, S., Fourie, P.H., 2010(a). The use of adjuvants to improve spray deposition and *Botrytis cinerea* control on Chardonnay grapevine leaves. Crop Protection 29: 58-67.

van Zyl, S.A., Brink, J.C., Calitz, F.J., Fourie, P.H., 2010(b). Effects of adjuvants on deposition efficiency of fenhexamid sprays to Chardonnay grapevine foliage. Crop Protection 29: 843-852.

Vicent, A., Armengol, J., García-Jiménez, J., 2009. Protectant activity of reduced concentration copper sprays against Alternaria brown spot on 'Fortune' mandarin fruit in Spain. Crop Protection 28: 1-6.

Whitney, J.D., Salyani, M., Chruchill, D.B., Knapp, J.L., Whiteside, J.O., Littell, R.C., 1989. A field investigation to examine the effects of sprayer type, ground speed, and volume rate on spray deposition in Florida citrus. Journal of agricultural Engineering Research 42: 275-283.

Table 1. Mean deposition quantity, uniformity, efficiency and quality realised by the water only control treatment (Water) and adjuvant treatments Break-Thru S240 (S240) and Break-Thru Union (Union) on leaves following sprays at 8, 14 and 20 L tree^{-1} to dense canopies in a 'Bahianina Araras' navel orchard (trial 1).

Treatment	Deposition quantity (FPC%)[a]			Deposition efficiency (FPC% normalised L tree^{-1})[a]		
	8 L tree^{-1}	**14 L tree^{-1}**	**20 L tree^{-1}**	**8 L tree^{-1}**	**14 L tree^{-1}**	**20 L tree^{-1}**
Water	4.06 cd	5.82 b	7.28 a	0.51 a	0.36 bc	0.42 ab
S240	3.61 d	4.09 cd	7.49 a	0.45 ab	0.37 bc	0.29 c
Union	3.46 d	5.05 bc	5.85 b	0.43 ab	0.29 c	0.36 bc
	Deposition Uniformity (CV% between leaves)[a]			**Deposition Quality (ICD%)[a]**		
Water	68.07 a	55.09 ab	57.33 ab	51.58 d	52.32 cd	55.03 bcd
S240	67.54 a	60.47 ab	57.86 ab	56.40 ab	55.82 abc	52.45 bcd
Union	58.47 ab	59.49 ab	52.39 b	54.12 bcd	59.16 a	56.37 ab

[a] For each parameter separately, values in each group of three columns for one variable followed by the same letter do not differ significantly (P > 0.05) according to Fisher's least significant difference test.

Table 2. Mean deposition quantity, uniformity, efficiency and quality realised by the water only control treatment (Water) and adjuvant treatments Break-Thru S240 (S240) and Break-Thru Union (Union) on leaves on the inside and outside of the dense tree canopies in a 'Bahianina Araras' navel orchard (trial 1).

Treatment	Deposition quantity (FPC%)[a]		Deposition efficiency (FPC% normalised L tree^{-1})[a]	
	Outer canopy	Inner canopy	Outer canopy	Inner canopy
Water	6.10 a	5.35 ab	0.46 a	0.40 ab
S240	5.39 ab	4.74 bc	0.41 a	0.33 c
Union	5.48 ab	4.07 c	0.43 a	0.29 c
	Deposition uniformity (CV% between leaves)[a]		**Deposition quality (ICD%)[a]**	
Water	57.93 ab	62.39 ab	53.31 bc	52.65 c
S240	58.53 ab	65.38 a	57.37 a	52.42 c
Union	55.31 b	58.36 ab	57.45 a	55.63 ab

[a] For each parameter separately, values in each pair of columns for one variable followed by the same letter do not differ significantly (P > 0.05) according to Fisher's least significant difference test.

Table 3. Mean deposition quantity, uniformity, efficiency and quality realised by the control treatment (Water) and adjuvant treatments Break-Thru S240 (S240) and Break-Thru Union (Union) on leaves at different spray volumes 8- ,14- and 20 L tree^{-1} in less dense canopies in a 'Palmer' navel orchard (trial 2).

Treatment	Deposition quantity (FPC%)[a]			Deposition efficiency (FPC% normalised L tree^{-1})[a]		
	8 L tree^{-1}	**14 L tree^{-1}**	**20 L tree^{-1}**	**8 L tree^{-1}**	**14 L tree^{-1}**	**20 L tree^{-1}**
Water	3.73 de	3.42 e	5.45 bc	0.47 b	0.24 d	0.27 d
S240	3.59 de	5.84 b	4.54 cde	0.45 b	0.42 bc	0.23 d
Union	4.66 cd	5.85 b	7.17 a	0.58 a	0.42 bc	0.36 c
	Deposition uniformity (CV% between leaves)[a]			**Deposition quality (ICD%)[a]**		
Water	72.80 a	71.42 a	60.39 bcd	56.47 ab	58.35 ab	56.76 ab
S240	65.18 ab	54.22 d	64.13 bcd	55.41 b	57.16 ab	57.82 ab
Union	61.31 bcd	64.13 abc	54.91 cd	57.71 ab	59.12 a	55.82 ab

[a] For each parameter separately, values in each group of three columns for one variable followed by the same letter do not differ significantly (P > 0.05) according to Fisher's least significant difference test.

Table 4. Mean deposition quantity, uniformity, efficiency and quality realised by the control treatment (water) and adjuvant treatments Break-Thru S240 (S240) and Break-Thru Union (Union) on leaves on the inside and outside of less dense canopies in a 'Palmer' navel orchard (trial 2).

Treatment	Deposition quantity (FPC%)[a]		Deposition efficiency (FPC% normalised L tree^{-1})[a]	
	Outer canopy	**Inner canopy**	**Outer canopy**	**Inner canopy**
Water	4.63 c	3.77 d	0.36 c	0.29 d
S240	4.78 bc	4.54 c	0.38 bc	0.35 c
Union	6.39 a	5.40 b	0.49 a	0.41 b
	Deposition uniformity (CV% between leaves)[a]		**Deposition quality (ICD%)[a]**	
Water	66.07 ab	70.35 a	60.98 ab	53.40 c
S240	61.66 bc	60.69 bc	58.79 b	54.80 c
Union	55.85 c	64.38 ab	61.77 a	53.33 c

[a] For each parameter separately, values in each pair of columns for one variable followed by the same letter do not differ significantly (P > 0.05) according to Fisher's least significant difference test.

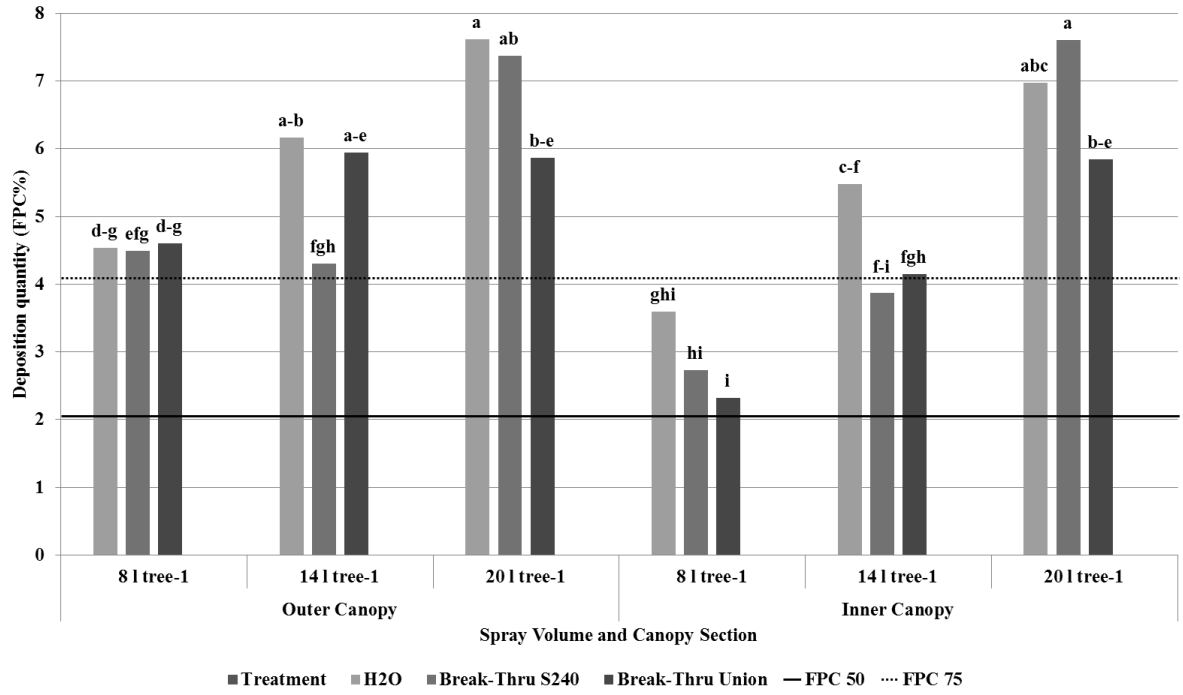

Figure 1. Mean deposition quantity realised by the water only control treatment (Water), Break-Thru S240 (S240) and Break-Thru Union (Union) on leaves following sprays at 8, 14 and 20 L tree^{-1} on the inside and outside of dense tree canopies in a 'Bahianina Araras' navel orange orchard when compared to FPC_{50} and FPC_{75} benchmarks at 2.07% and 4.14%, respectively (Trial one).

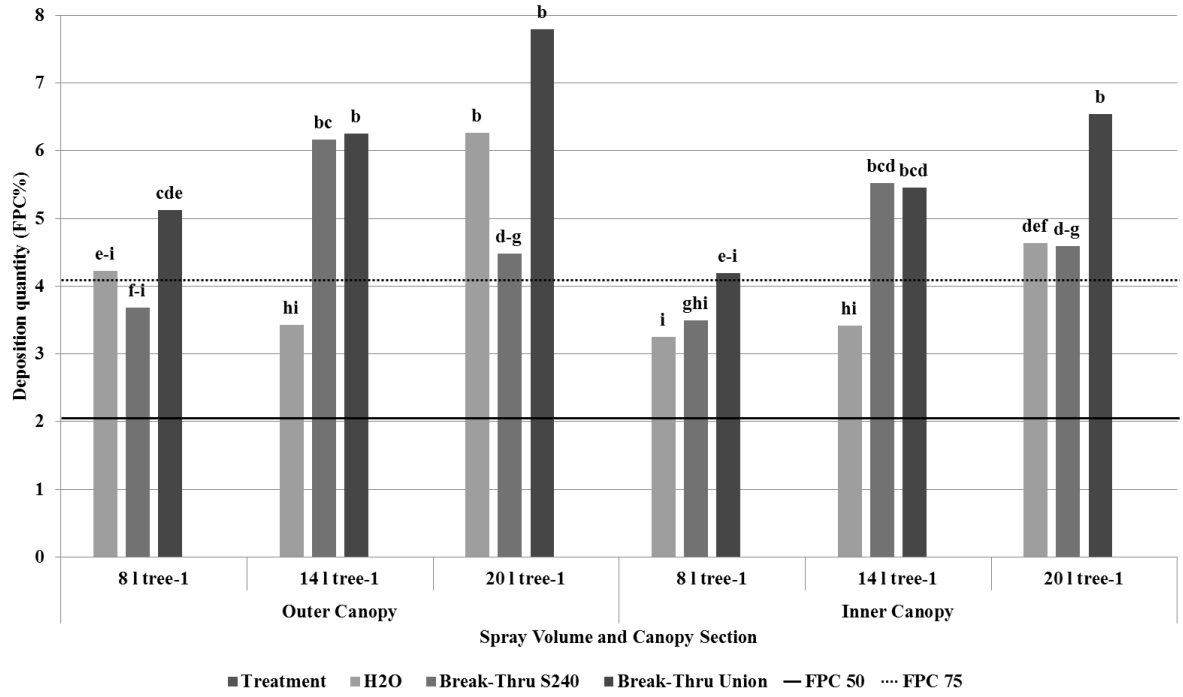

Figure 2. Mean deposition quantity realised by the water only control treatment (Water), Break-Thru S240 (S240) and Break-Thru Union (Union) on leaves following sprays at 8 ,14 and 20 L tree^{-1} on the inside and outside of pruned, less dense canopies in a 'Palmer' navel orange orchard when compared to FPC_{50} and FPC_{75} benchmarks at 2.07% and 4.14%, respectively (Trial two).

CHAPTER 4

Field evaluation of selected spray adjuvants in Southern African citrus orchards

ABSTRACT

Adjuvants are regularly included in crop protection sprays in citrus production in South Africa. The influence of adjuvants on deposition parameters and how this influences disease control at high spray volumes was unknown. Commercially available adjuvants [Break-Thru S240 (organosilicone), Nu-Film-17 (terpene oil), Citrole100 (paraffinic oil complex), Villa51 (alkylpolyethene glycol ether), Wetcit (inorganic compound), Entrée and Exit (vegetable oil)] were evaluated in three orchard spray trials. Trial 1 evaluated deposition on lemon and grapefruit trees using a multi-fan air blast tower sprayer at 2 km h^{-1} and spray volume of 4232 L ha^{-1}. Other trials evaluated deposition parameters at medium and high spray volumes using a multi-fan (trials 2) or air blast tower sprayer (trial 3). Volumes were manipulated through forward speed: trial 2: medium spray volumes 3430 L ha^{-1} at 3.4 km h^{-1} and 3786 L ha^{-1} at 3.2 km h^{-1}; high spray volumes 6860 L ha^{-1} at 1.7 km h^{-1} and 7535 L ha^{-1} at 1.6 km h^{-1}; trial 3: 5411 L ha^{-1} at 4.5 km h^{-1} and 10389 L ha^{-1} at 2.5 km h^{-1}. In each trial, 10 trees were sprayed from both sides with a spray mixture containing a yellow fluorescent pigment (1 mL L^{-1}) with or without aforementioned adjuvants at recommended dose rates. Leaves were sampled from six positions (inner and outer canopy position at the top, middle and bottom of the tree) from three randomly selected trees. Deposition quantity and quality of fluorescent pigment was determined on upper and lower leaf surfaces of leaves using fluorometry, digital photomacrography and image analyses. Spray uniformity between leaves and spray efficiency (deposition quantity per 1000 L spray volume) were also compared between treatments. In trial 1, Break-Thru S240 and Nu-Film-17 realised better deposition quantity, quality, uniformity and canopy penetration than the water only control treatment. Deposition quantity in the other two trials was generally higher at higher spray volumes, but spray efficiency was significantly better at lower spray volumes. In trial 2, adjuvants generally had similar or poorer deposition parameters as the control, and similar or improved deposition in trial 3. In general, the best and more consistently performing adjuvants were the Break-Thru S240 and Nu-Film-17, followed by the Citrole100; performance of Entreé, Exit, Villa51 and Wetcit was inconsistent. Suboptimal and irregular performance by adjuvants were ascribed to high spray volumes used and/or too high adjuvant concentration, which led to increased levels of run-off and poor deposition parameters.

INTRODUCTION

Medium to high volume, dilute fungicide sprays ranging from 6000 to 10 000 L ha^{-1} forms the basis of conventional fungal disease control in South African citrus production (Grout, 1997, 2003). This is markedly higher than spray volumes used in other citrus producing countries, for example Spain (1000 to 5000 L ha^{-1}) (Garcerá *et al*., 2014; 2017) and the United States of America (200 to 4500 L ha^{-1}, in some cases up to 7000 L ha^{-1}) (Stover and Salvatore, 2002; Salyani and Farooq, 2005; Salyani *et al*., 2007; Salyani, 2015). It is plausible that higher spray volumes for fungal disease control has largely evolved unintentionally from control methods developed for the control of Californian red scale (*Aonidiella aurantii* (Maskell)) through medium to high volume mineral/petroleum based oil, pesticide and oil combination sprays using hand lances following to the development of organophosphate pesticide resistance in the 1980's in South Africa. Additionally, the transition from pressurised hand lance sprays to tractor drawn spray machines also resulted in growers trying to duplicate the same degree of 'wetting' or 'cover' with the tractor drawn machines as was obtained with hand lances, even though the modern sprayers are capable of producing effective spray plumes with various nozzle and fan technologies at lower spray application volumes.

Producers need to control important fungal pathogens of which citrus black spot (CBS) (*Phyllosticta citricarpa* (McAlpine) van der Aa (syn. *Guignardia citricarpa* Kiely)) is the most important due to its impact on phytosanitary trade (CBS Expert Panel, 2013, 2014, 2015; EFSA, 2014). Medium to high volume fungicide spray application provides cost-effective control of CBS (Kotzé, 1981; 2000; Schutte *et al*., 1997) and other diseases like Alternaria brown spot (*Alternaria alternata* (Fr: Fr) Keissl, tangerine pathotype) (Schutte, 1996). These conventional application methods are also popular among producers since it serves as a buffer for loss of efficacy due poor canopy preparation in generally large and dense canopies, operator error and the use of poor equipment and application technique. However, this methodology is super-optimal, costly and not efficient in terms of time and input costs causing high product losses due to exo- and endo-drift (Salyani and Farooq, 2005; Cunha *et al*., 2012; Salyani *et al*., 2013; van Zyl *et al*., 2014). Furthermore, market and public pressure worldwide are demanding reduced plant protection product (PPP) use that is only achievable through improvement of application technology and strategies.

Spray adjuvants offer potential to improve PPP deposition quantity and quality on target surfaces and therewith improved disease and pest control (van Zyl *et al*., 2010a). A wide range of adjuvant formulations is available for use in PPP spray application in citrus orchards. However, they are often used with little to no research on the effect of adjuvants on deposition parameters

at these medium to high application volumes and if there is any beneficial effect at all (Steurbaut, 1993). Adjuvants are generally added to spray mixtures with the assumption that it would improve deposition parameters of the plant protection product used on the target surface (be it the leaves, twigs or fruit) and therefore improve pest or disease control as was observed in various studies (Butler Ellis and Tuck, 1999; Gent *et al*., 2003; Gaskin *et al*., 2004; Green and Beestman, 2007; van Zyl *et al*., 2010 a,b). Adjuvants achieve improved deposition by reducing the surface tension of spray droplets and/or by modifying the surface characteristics of the target surface, influencing the droplet impact, wetting, spreading and dispersing effect of the sprayed mixture and therefore the distribution of the active ingredient (Knoche *et al*., 1992; Hazen, 2000). Furthermore, some of the sticker formulations have the ability to improve adherence and weathering of the active ingredient on the target surface (Hazen, 2000; Faers and Pontzen, 2008). Adjuvant formulation also influences droplet formation (Butler Ellis *et al*., 1997; Butler Ellis and Tuck, 1999) and therefore deposition parameters (van Zyl *et al*., 2014).

Thus, adjuvants can be a tool to improve spray application in citrus but have to be evaluated in order to recommend the optimal application parameters. On other crops, various adjuvant formulation types have been studied with varying results. Van Zyl *et al*. (2010a, 2010b) demonstrated the beneficial effect that certain adjuvant formulations can have on deposition parameters if used at the correct application volume and dosage on grapevine leaves. Furthermore, van Zyl *et al*. (2010a) indicated that improved deposition of fungicides had the potential to improve the control of *Botrytis cinerea* on grapevine leaves. The beneficial effect of adjuvant use have also been evaluated on various other crop types, including avocados, kiwifruit, broad bean and cabbage (Gaskin *et al*., 2004; 2006; Gaskin and Steele, 2009).

Cunningham and Harden (1998; 1999) indicated the potential and effectiveness of spray deposition using reduced volume sprays in citrus orchards. Van Zyl *et al*. (2014) demonstrated and indicated the potential of reduced spray volumes using organo-modified trisiloxanes or organosilicones as tank mix adjuvants at a range of spray volumes in South African citrus orchards.

The objective of this study was to evaluate how various commonly used adjuvants influenced foliar deposition parameters at different spray application volumes with conventional spray machines in commercial citrus orchards. This was done using a previously developed deposition assessment protocol consisting of fluorometry, digital photomacrography and image analysis (Brink *et al*., 2004, 2006; Fourie *et al*., 2009; Schutte *et al*., 2012; van Zyl *et al*., 2014).

MATERIALS AND METHODS

Spray application

Trial 1: Lemon and grapefruit orchards

The trial was conducted in visually uniform (areas where trees have similar canopy characteristics e.g. height, width and density) sections of a 'Star Ruby' grapefruit orchard (*Citrus sinensis* (L.) Osbeck) [2.7×2.9 m trees (H×W); 6×2 m inter-row spacing] and repeated in a Eureka lemon (*Citrus sinensis* (L.) Osbeck) [3.6×2.7 m trees (H×W); 6×2 m inter-row spacing] orchard near Hoedspruit (Limpopo province, South Africa) in February 2009. Canopy density was visually assessed on a 5-point scale (van Zyl *et al*., 2014), with 1 being orchards with very sparse heavily aerated canopies and 5 being orchards with very dense, poorly aerated canopies with no pruned windows. The Star Ruby trial section was rated as a 4.5 and the Eureka lemon as a 4 in terms of canopy density.

For each treatment combination, a single row-section with 10 trees was marked and sprayed from both sides with a tractor-drawn, power take-off (PTO) powered, multi-fan air blast BSF-Multiwing sprayer (BSF Hoedspruit, South Africa). The spray applicator is a high profile (tower) sprayer with 22 nozzle ports per side, with air being generated by five fans per side positioned vertically behind the nozzles without ducting on the sprayer tower. A high profile sprayer was used to match canopy height and to negate the effects of using a low profile applicator in large canopies (Cunningham and Harden, 1998; van Zyl *et al*., 2014). A combination of hollow and full cone nozzles was used (Jacto Disc-Core type J4-3 full cone and J5-2 hollow cone nozzle combination) at 540 rpm PTO speed, 1000 KPa pressure at 2 km h^{-1} tractor speed to realise a spray volume of 4232 L ha^{-1}. Sprays consisted of fluorescent pigment (Yellow Fluorescent Pigment 40% EC; South Australian Research and Development Institute (SARDI); Loxton, South Australia; 1 mL L^{-1}) alone (control treatment with no adjuvant) or with selected spray adjuvants at the registered industry application rates for citrus (Table 1). Two buffer rows were left unsprayed between treatments. The spray tank, spray nozzles, filter and pipes of the spray machine was thoroughly washed and flushed with water after each treatment.

Trials 2 and 3: Navel orchards

Trial 2 was conducted in uniform sections of 'Bahianina' navel (*Citrus sinensis* (L.) Osbeck) [3.4×3.4 m trees (H×W) with density of 3 on a 5-point scale; 3×5.5 m inter-row spacing] orchard near Clanwilliam (Western Cape Province, South Africa) in November 2009. Trial lay-out was similar to that explained for Trial 1. A BSF-Multiwing sprayer with a nozzle combination [Jacto Disc-Core type J4-3 full cone and J5-2 hollow cone nozzles combination) was again used, but at

a spray pressure of 1500 KPa, PTO speed of 540 rpm and at two tractor speeds: 1.7 km h^{-1} realising a spray volume of 6860 L ha^{-1} (high volume application) and 3.4 km h^{-1} realising 3430 L ha^{-1} (medium volume application). A repeat was sprayed in the same 'Bahianina' navel orchard on a different set of uniform trees [3.9×3.7 m trees (H×W) with density of 4 on a 5-point scale; 3×5.5 m inter-row spacing] in October 2010. Trial layout, sprayer, nozzle selection and spray pressure was similar to that of the previous trial, only differing in tractor speeds: 1.6 km h^{-1} resulting in 7535 L ha^{-1} (high volume application) and 3.2 km h^{-1} resulting in 3768 L ha^{-1} (medium volume application). Treatments were similar to those of previous trials except for Herbiplus, which was replaced by Citrole100, and Exit, which was replaced by Entreé (Table 1).

Trial 3 was conducted in a 'Cara Cara' navel (*Citrus sinensis* (L.) Osbeck) orchard [4.2×4.3 m trees (H×W) with density of 4.3 on a 5-point scale; 3.5×7 m inter-row spacing] in Schoemanskloof near Nelspruit (Mpumalanga province, South Africa). Trial layout was similar to previous trials, but an Ultima air blast tower sprayer was used (Johnson Advanced Machinery; www.citro.co.za). The Ultima sprayer is an axial fan, high profile sprayer with a 6-m pressurized tower. Air is ducted out through a narrow slit on either side of the pressurised tower. Thirty nozzle ports are attached to an oscillating boom on each side. A nozzle combination (TeeJet Disc-Core type D4-DC56 full cone and D6-DC45 hollow cone nozzle combination) at 1500 KPa pressure, 540 rpm PTO speed were used at two tractor speeds: 2.5 km h^{-1} realising a spray volume of 10389 L ha^{-1} (very high volume application) and at 4.8 km h^{-1} realising 5411 L ha^{-1} (high volume application). Adjuvant treatments were the same as in Trial 2. Trial 3 was not repeated.

Sampling of all field evaluations

The 10-tree sprayed section was an experimental unit. As sub-samples, three uniform trees were selected from each 10-tree sprayed section. From each tree, twelve randomly selected intact leaves were carefully sampled after the spray mixture has dried from each of the six positions in the tree canopy for spray deposition analysis. The six positions were inner (30 to 50 cm into the tree) and outer canopy (leaves on the outside of the tree) at the upper, middle and lower parts of each of the selected trees. Leaves picked from these six positions were collected and stored separately in marked polyethylene bags. Stored leaves were transported to the laboratory under cool, dry conditions where it was stored at 4°C until further analysis.

Spray deposition analysis

For deposition analysis, petioles were removed from leaves at the base of the leaf blade using a pair of scissors. A single leaf was positioned in the middle of a back-illuminated red Perspex box

(300×210×110 mm) inside a dark room to reduce any shadowing and to enhance edging of leaves in captured images during analysis. The leaf was covered with a glass pane (200×200×2 mm) and illuminated using an ultra-violet light source (UV-A at ≈ 365 nm; Labino Mid-Light; www.labino.com). Digital photos were taken in Canon RAW file format (*.CR2 ≈ 10 MB) of the upper and lower leaf surfaces using a Canon EOS 40D camera equipped with a 60 mm macro lens. The camera was attached to a tripod in a fixed position directly above the leaf. RAW image files were converted to 8-bit Exif-TIFF files (*.TIF ≈ 30 MB using Digital Photo Professional version 4.0, CANON INC.; www.canon.com) for digital image analysis to determine the deposition parameters (van Zyl *et al*., 2013, 2014).

Spray deposition assessment involved digital image analysis (Image Pro Plus software version 7.0, Media Cybernetics, www.mediacy.com) to determine the deposition quantity and quality per leaf. Similar to the methodology used in van Zyl *et al*. (2013, 2014), deposition quantity was measured as percent total leaf area covered by pigment particles (percentage fluorescent particle coverage; FPC%) (van Zyl *et al.*, 2013). Deposition data were compared to the FPC benchmarks modelled by van Zyl *et al*. (2013). The FPC_{50} (2.07 FPC%) and FPC_{75} (4.14 FPC%) benchmarks indicate 50% and 75% control of Alternaria brown spot on mandarin leaves, respectively. For the deposition quality assessment, the leaf area was divided into equally-sized squares [100 × 100 pixels (10000 pixels)] (van Zyl *et al.*, 2014). Depending on the leaf size, this amounted to at least 20 to >250 individual squares per leaf, of which the percent area covered by fluorescent pigment particles was determined for each square. The Interquartile Coefficient of Dispersion (ICD%), a form of the coefficient of quartile variation (Bonnet, 2006) per leaf [((3rd quartile – 1st quartile)/(3rd quartile + 1st quartile))*100], was used as a measure of deposition quality per leaf, *i.e.* uniformity of deposition on the leaf surface. Lower interquartile coefficient of dispersion values were indicative of better deposition quality (van Zyl *et al*., 2014). Deposition uniformity between leaves was calculated as the coefficient of variation (CV%) in pigment deposition in a 12-leaf batch (standard deviation × 100/mean). Deposition efficiency of treatments with various spray volumes was also analysed as deposition quantity normalised to a spray volume of 1000 L ha^{-1} (FPC% per 1000 L ha^{-1}).

Statistical analysis

A completely randomised split plot design with treatment as main plot factor, each tree sampled as sub-sample, position within each tree canopy as subplot factor and leaf surface (upper/lower) as sub-subplot factor was used. In order to simplify and increase robustness of results, the vertical canopy position factor was ignored, unless meaningful conclusions could be drawn. Median

deposition quantity (FPC%), quality (ICD%), and uniformity (CV%) and efficiency (FPC% per 1000 L ha^{-1}; only for trials where spray volumes varied) data were subjected to appropriate analysis of variance (ANOVA). Spray efficiency was used to evaluate normalised deposition quantity data specific for treatment and volume; other factors was ignored in that analysis. For trial 1, lemon and grapefruit orchard data were combined to serve as repeats. For trial 2, data from Clanwilliam sprays in 2009 and 2010 were combined with spray volumes classified as high and medium spray volumes to serve as repeats. Trial 3 was not repeated and analysed separately. Student's T-test for least significant difference was used to identify significant differences between treatments at a confidence interval of 95%. Data from upper and lower leaf surfaces was analysed separately but were combined when describing the results. SAS version 8.2 statistical software (SAS institute Inc., 1999) was used for analysis.

RESULTS

Since high profile sprayers were used in each trial, discussion of vertical canopy position was ignored if no noteworthy effects or interactions were observed.

Trial 1: Lemon and grapefruit orchards

Deposition quantity

Analysis of variance of deposition quantity data (FPC%) indicated a meaningful interaction for treatment × horizontal canopy position ($P = 0.0942$). Significantly higher deposition quantity levels were generally observed on outer than on inner canopy leaves. On outer canopy leaves, the highest deposition quantity was realised by Break-Thru S240 (3.84 FPC%) and Nu-Film-17 (3.72 FPC%), significantly more than all other treatments. Herbiplus (2.76 FPC%) and Villa51 (2.70 FPC%) realised similar deposition quantities than the control treatment (2.94 FPC%). Exit realised the lowest deposition quantity on outer canopy leaves (1.68 FPC%), significantly lower than all other treatments except for Wetcit (2.22 FPC%) (Table 2). On inner canopy leaves, a similar trend was observed with Break-Thru S240 (2.19 FPC%) and Nu-Film-17 (2.19 FPC%) realising the highest deposition quantity, significantly more than the other treatments. The control treatment (1.27 FPC%) did not differ significantly from Herbiplus (1.55 FPC%), Wetcit (1.47 FPC%), Villa51 (1.33 FPC%), and Exit (0.84 FPC%) (Table 2).

The ratio between deposition quantity on outer and inner canopy leaves indicated that all adjuvant treatments (49 to 66%) improved canopy penetration compared to the control treatment (43%). Wetcit had the best penetration ratio of 66% (Table 2).

Deposition quantity results were compared to the FPC benchmarks [FPC_{50} (2.07 FPC%) and FPC_{75} (4.14 FPC%) benchmarks indicate 50% and 75% control of Alternaria brown spot on mandarin leaves, respectively] (Van Zyl *et al*., 2013). On outer canopy leaves, all treatments realised deposition quantity between the FPC_{75} and FPC_{50} benchmark with Exit being the only exception with a deposition quantity below the FPC_{50} benchmark. On inner canopy leaves, only Break-Thru S240 and Nu-Film-17 had deposition quantity above the FPC_{50} with the rest of the treatments, including the control, deposition well below the FPC_{50} benchmark (Table 2).

Deposition quantity was generally significantly ($P < 0.0001$) higher in top and middle (2.45 and 2.27 FPC%) than bottom (1.86 FPC%) of tree canopies.

Deposition uniformity

Analysis of variance of deposition uniformity data (CV%) indicated no meaningful or significant interactions ($P < 0.05$), but significant main effects for horizontal canopy position ($P < 0.0001$) and vertical canopy position ($P = 0.0383$) whilst a meaningful interaction was observed for treatment ($P = 0.0686$). Deposition uniformity was better (lower CV%) on outer canopy leaves than on inner canopy leaves (57.71 *vs.* 75.94%). Deposition uniformity was poorer amongst bottom canopy leaves (71.66 CV%) than top (62.54 CV%) and middle canopy leaves (66.25 CV%) canopy leaves. Nu-Film-17 (62.92 CV%), Herbiplus (62.18 CV%) and Wetcit (62.13 CV%) realised significantly better deposition uniformity than the control treatment (73.72 CV%) whilst Break-Thru S20 (66.37 CV%), Villa51 (65.34 CV%) and Exit (75.10 CV%) realised similar uniformity to the control treatment (Table 3).

Deposition quality

Analysis of variance of deposition quality (ICD%) indicated no meaningful or significant interactions ($P < 0.05$), but significant main effects for treatment ($P = 0.0018$) and vertical canopy position ($P < 0.0001$). The least variation in deposition (best deposition quality) was observed on top canopy leaves (50.24 ICD%), significantly better than that found on middle (53.72 ICD%) and bottom canopy leaves (58.37 ICD%). The best deposition quality was realised by Break-Thru S240 (46.14 ICD%), significantly better than deposition quality following the water only control treatment (52.23 ICD%). Nu-Film-17, Villa51 and Herbiplus (47.73 to 55.17 ICD%) realised statistically similar deposition quality than the control treatment, whilst Wetcit (59.07 ICD% and Exit (64.89 ICD%) realised significantly poorer deposition quality than the control treatment (Table 3).

Trial 2: 'Bahianina' navel orchards

Deposition quantity

Analysis of variance of deposition quantity (FPC%) indicated no significant interactions ($P < 0.05$), but significant main effects for spray volume ($P = 0.0025$), horizontal canopy position ($P < 0.0001$) and vertical canopy position ($P = 0.0173$). Deposition quantity realised was significantly higher at the high volume than the medium volume sprays (2.75 *vs.* 1.79 FPC%). Deposition quantity was higher on outer canopy leaves than on inner canopy leaves (2.47 *vs*. 2.06 FPC%) and deposition quantity realised on top canopy leaves was significantly higher than that realised on middle and bottom canopy leaves (2.42 *vs.* 2.20 and 2.18 FPC%).

Although not significant, the treatment × volume × horizontal canopy position interaction ($P = 0.7720$) was discussed to highlight treatment effects as influenced by spray volume and horizontal canopy position. At the high spray volume, the water only control treatment and Break-Thru S240 realised the highest spray deposition quantities on outer and inner canopies: 3.91 and 3.12 FPC%, and 3.34 and 3.26 FPC%, respectively (Table 4). Nu-Film-17 performed similarly to Break-Thru S240 on inner and outer canopy canopy leaves (3.06 and 2.76 FPC%, respectively), whilst Break-Thru S240 had a better penetration ratio (98% *vs.* 90%). The rest of the adjuvant treatments realised significantly poorer deposition quantity than the control treatment (2.75 to 1.88 FPC% on outer canopy and 2.58 to 1.61 FPC% on inner canopy leaves). Exit had the poorest deposition quantity on inner and pouter canopy leaves (1.88 to 1.61 FPC%), significantly lower than all treatments except for Entreé (2.39 to 1.96 FPC%). As indicated by the Outer:Inner ratios, the adjuvant treatments effected similar or improved spray penetration in the canopy compared with the control treatment (98 to 82% *vs*. 80%). (Table 4).

At the medium spray volumes, no differences were observed between the water only control (2.57 and 2.26 FPC%) and adjuvant treatments (2.03-2.10 and 1.46-1.77 FPC%) on inner and outer canopy leaves except for Entrée (1.06 and 0.44 FPC%) and Exit (1.42 and 1.18 FPC%), which performed markedly poorer (Table 4). Canopy penetration ratios following the medium volume sprays indicated that most of the adjuvant treatments resulted in similar or poorer canopy penetration in relation to the control treatment (72 to 84% *vs.* 88%), with Entreé realising a very poor penetration ratio of 42%.

Deposition quantity results were compared to the FPC benchmarks (Van Zyl *et al*., 2013). None of the treatments realised deposition quantity above the FPC_{75} (4.14 FPC%). At the high spray volume, all treatments, except Exit and Entreé (on inner canopy leaves) realised deposition quantity above the FPC_{50} (2.07 FPC%). At the medium spray volume, the water only control treatment realised deposition above the FPC_{50}. Most of the adjuvant treatments (except Entrée

and Exit) realised deposition similar to the FPC_{50} on outer canopy leaves. On inner canopy leaves, none of the adjuvants had deposition quantities above the FPC_{50}.

Deposition efficiency values (FPC% per 1000 L spray volume) for medium and high volume sprays for each treatment did not differ significantly, but were generally markedly better at medium spray volumes (21 to 76%) than high spray volumes for most treatments, except Break-Thru S240 (7%) and Entrée (-23%) (results not shown).

Deposition uniformity

Analysis of variance of deposition uniformity (CV%) between leaves indicated no significant or meaningful interactions. Significant main effects were observed for spray volume (P = 0.0006), horizontal canopy position (P < 0.0001) and vertical canopy position (P = 0.0001). Better deposition uniformity was observed between leaves following the high volume sprays (59.23 CV% *vs.* 70.68 CV% for the low volume sprays). Deposition uniformity was significantly better on outer canopy leaves than on inner canopy leaves (60.88 *vs.* 69.04 CV%). Deposition uniformity on middle and bottom canopy leaves were significantly better than that realised on top canopy leaves (61.56 and 63.97 *vs.* 69.34 CV%). Although treatment was not significant as main effect (P = 0.2529), Villa51 had significantly better deposition uniformity than Entrée (58.82 *vs.* 75.34 CV%), with the remainder of adjuvants yielding intermediate and similar uniformity levels (62.28 to 71.04 CV%; results not shown).

Deposition quality

The Nu-Film-17 treatment was not included in the analysis due to loss of data. Analysis of variance indicated no significant or meaningful interactions (P > 0.05), no treatment effect (P = 0.8032), but did indicate a significant horizontal canopy position effect (P < 0.0001). Significantly better deposition quality was generally observed on inner canopy leaves than outer canopy leaves (55.91 *vs.* 58.85 ICD%) (results not shown).

Trial 3: 'Cara Cara' navel orchard

Deposition quantity

Analysis of variance of deposition quantity data (FPC%) indicated a significant treatment × volume × vertical canopy position × horizontal canopy position (P < 0.0182) interaction. Due to the complexity of the interaction it was not discussed. To present results in a similar and comparable manner as for trial 2, the treatment × volume × horizontal canopy position interaction (P = 0.4400) was discussed. At 10389 L ha^{-1} significantly higher deposition quantities were generally realised

on outer canopy leaves than on inner canopy leaves. On outer canopy leaves, Nu-Film-17 realised the highest deposition quantity (8.13 FPC%), significantly higher than all other treatments (4.93 to 6.32 FPC%). Break-Thru S240 (6.30 FPC%) and Entreé (6.32 FPC%) realised significantly higher deposition quantity than the control treatment (4.90 FPC%), whilst Villa51, Wetcit and Citrole100 (4.93, 5.35 and 5.40 FPC% respectively) realised similar deposition than the control treatment. On inner canopy leaves, Nu-Film-17 (3.88 FPC%) and Wetcit (3.94 FPC%) realised significantly higher deposition quantity than the control treatment (2.86 FPC%). The rest of the adjuvant treatments (2.88 to 3.35 FPC%) realised similar deposition quantity to the control treatment (Table 5). Inner:Outer ratios indicated that only Wetcit (74%) markedly improved canopy penetration relative to the control treatment, whilst the other adjuvants had similar or poorer penetration ratios (48 to 61%) (Table 5).

At 5411 L ha^{-1} higher deposition quantities were also realised on outer canopy leaves than on inner canopy leaves. Nu-Film-17 had significantly higher deposition quantity (5.28 FPC) than all other treatments. The rest of the adjuvants (3.84 to 4.45 FPC%) except for Villa51 (3.66 FPC%) realised significantly higher deposition quantity than the control treatment (3.00 FPC%). On inner canopy leaves, only Wetcit (2.18 FPC%) realised significantly higher deposition quantity than the control treatment (1.37% FPC%), with the rest of the treatments realising similar quantities (1.07 to 1.63 FPC%) (Table 5). Canopy penetration ratios indicated that only Wetcit (54%) improved canopy penetration relative to the control treatment (46%), while the other adjuvants had similar or poorer ratios (26 to 45%) (Table 5).

At the high spray volume, deposition quantities were above the FPC_{75} benchmark on outer canopy leaves and above the FPC_{50} benchmark on inner canopy leaves for all treatments. At the lower spray volume, only Break-Thru S240 and Nu-Film-17 had deposition quantities above FPC_{75} on outer canopy leaves, with the other treatment landing quantities above FPC_{50}. On inner canopy leaves, deposition quantities were lower and only Wetcit had levels above FPC_{50}.

Vertical canopy position had a significant effect ($P < 0.0001$) on deposition quantity, with more pigment retained at lower canopy positions: bottom (4.33 FPC%), mid (3.94 FPC%) and top (2.92 FPC%) canopy positions.

Deposition efficiency (FPC% per 1000 L spray volume) measured on outer canopy leaves was generally significantly better (17 to 44%) at 5411 than 10389 L ha^{-1}. However, on inner canopy leaves the efficiency levels were generally similar or poorer (10% to -38%) (results not shown).

Deposition uniformity

Analysis of variance of deposition uniformity between leaves indicated a significant treatment × vertical canopy position × horizontal canopy position (P = 0.0252) and treatment × volume interaction (P = 0.0231). The significance of the former interaction was not meaningful and for better interpretation of deposition uniformity, the treatment × volume interaction was discussed (Table 6). At 10389 L ha^{-1}, all the adjuvant treatments had significantly better deposition uniformity (50.05 to 56.91 CV%) than the water only control treatment (68.19 CV%), except for Villa51 (58.57 CV%), which had similar uniformity than the control treatment. At 5411 L ha^{-1}, Villa51 (61.16 CV%) and Citrole100 (65.56 CV%) had significantly better uniformity than Entreé (76.62 CV%), with the other adjuvant treatments resulting in intermediate levels (71.27 to 71.48 CV%), similar to the control treatment (69.24 FPC%) (Table 6).

Deposition quality

Analysis of variance of deposition quality (ICD%) indicated a significant interaction for treatment × volume × horizontal canopy position (P = 0.0267). At 10389 L ha^{-1} Break-Thru S240, Nu-Film-17, Citrole100 and Wetcit (49.85 – 52.12 ICD%) realised significantly better deposition quality on outer canopy leaves than the control treatment (57.51 ICD%). On inner canopy leaves, these adjuvants (48.78 - 54.50 ICD%) also realised significantly better deposition quality than the control treatment (60.11 ICD%). Villa51 and Entreé did not improve deposition quality significantly on outer or inner canopy leaves compared with the control treatment (results not shown). At 5411 L ha^{-1}, deposition quality was generally better on outer canopy leaves than on inner canopy leaves. On outer canopy leaves, only Nu-Film-17 (46.43 ICD%) realised better deposition quality than the control treatment (56.29 ICD%), with the other adjuvants with values similar to the control (51.10 to 54.82 ICD%). On inner canopy leaves, all adjuvants realised similar variation in pigment distribution than the control treatment (59.14 ICD%) with the exception of Villa51 (66.29 ICD%), Entreé (75.90 ICD%) and Citrole100 (68.29 ICD%), which realised significantly poorer deposition quality (results not shown).

DISCUSSION

This study evaluated the influence of different spray adjuvants on deposition parameters throughout citrus tree canopies on different citrus types/cultivars, at different spray volumes and calibration settings. In previous field- (Schutte *et al*., 2012; van Zyl *et al*., 2014) and laboratory (van Zyl *et al*., 2013) trials it was found that the use of fluorometry, photomacrography and digital image analysis as employed in this study proved to be a suitable tool to evaluate deposition

parameters on citrus trees. Furthermore, the implementation of deposition quantity benchmarks, for the biological interpretation of deposition results, proved to be insightful in evaluating deposition results (van Zyl *et al*., 2014).

Deposition quantity values were always higher on outer canopy leaves than on inner canopy leaves, and uniformity and quality values were also better. The latter deposition parameters were generally poorer at low spray volumes (higher forward speed) than at high spray volumes (slower forward speed). This concurs to various other studies (Farooq and Salyani, 2002; Khot *et al*., 2012; Dekeyser *et al*., 2014; van Zyl *et al*., 2014). These findings also concur with results on artificial trees where foliar deposition decreased with increase in canopy depth (Dekeyser *et al*., 2014). Khot *et al.* (2012) evaluated deposition on artificial targets and citrus leaves as influenced by a specialised sprayer at different air assistance levels in small citrus trees and also found that deposition decreased as canopy depth increased. Salyani and Whitney (1990) and Whitney *et al.* (1989) found that deposition quantity and variation in deposition (uniformity) decreased with citrus canopy depth and density. As citrus trees (and other row crops such as grapes, tomatoes, apples and pears) become deeper/denser, the path of the droplet from the nozzle/sprayer becomes more complex/obstructed and is therefore less likely to land targets on the inside of the canopy. Sprayer airflow characteristics (speed and volume), forward travel speed and droplet size spectrum will thus have a direct effect on canopy penetration (Dekeyser *et al.,* 2014; Khot *et al.,* 2012).

Deposition parameters also varied between upper, middle and lower canopy leaves, with significant differences in some cases. In trials 1 and 2, better deposition quantity was realised on top than middle and bottom canopy leaves, whilst deposition uniformity was better in upper and lower tree canopies in trial 1 and 2, respectively. In trial 3, using a different spray machine and generally higher spray volumes, deposition quantity and uniformity were better in lower tree canopies. These differences could not be ascribed to the addition of adjuvant treatments, but rather to canopy geometry, sprayer profile height, application technique and calibration. This illustrates the importance of using high profile spray machines in large three-dimensional crops. In previous research (Cunningham and Harden, 1998, Khot *et al*., 2012; P.H Fourie, unpublished results), low profile sprayers retained lower deposition quantity in tops of trees, as well as concomitant variation in deposition quality and uniformity. This phenomenon is due to the increased distance droplets have to travel within the air column to reach the top of canopies. Thus, droplets are exposed longer to environmental conditions such as temperature, wind and humidity, decreasing droplet lifespan. Furthermore, droplets reaching the top of canopies need to penetrate the complex leaf, fruit and twig complex with poorer momentum due to travel time and distance. Although not measured, variation in deposition parameters can further be ascribed to

variation in air speed along the height of the spray tower and the height of the tower as influenced by sprayer design and various calibration settings (such as tractor speed), whilst canopy characteristics also influenced penetration and deposition in tops of trees, which are typically further removed from the tower than the middle and lower canopy sections (Cunningham and Harden, 1998; Khot *et al*., 2012; Dekeyser *et al*., 2014; Duga *et al*., 2015).

The addition of adjuvants can affect the quality of the spray produced by spray nozzles (Miller and Ellis, 1997) and droplet size spectrum (Holloway, 1994). In this study, the addition of certain adjuvants improved deposition quantity, quality and uniformity on inner canopy leaves (though not significantly in some cases), as was also indicated by Outer:Inner penetration ratios, which were improved by adjuvants in all trials, except at the lower spray volumes in trials 2 and 3. The latter observation might be attributed to generally poorer spray penetration observed at faster tractor speeds (this study; Fourie *et al.*, unpublished). This concurs with what was found by van Zyl *et al.* (2014) that adjuvants were more beneficial when canopies were spray-friendly, *i.e.* less dense.

There was no clear “best adjuvant performer” throughout the four trials sprayed with results varying between trials. Deposition quantity, uniformity and quality were only improved in certain cases by certain adjuvants. In trial 1 on lemon and grapefruit trees (sprayed at 4232 L ha^{-1}), only Break-Thru S240 and Nu-Film-17 improved deposition quantity and quality in relation to the control treatment on inner and outer canopy leaves with deposition uniformity being improved by Nu-Film-17, Herbiplus and Wetcit. In trial 2 on navel orange trees, none of the adjuvants improved deposition quantity in relation to the control treatment at both spray high and medium spray volumes, and most adjuvants (except Entrée and Exit) performed similarly. In trial 3, all adjuvants performed better than the control treatment, improving deposition quantity, significantly in some cases, on inner and outer canopy leaves at the high volumes evaluated (10389 and 5411 L ha^{-1}).

Higher spray volumes generally realised higher deposition quantity and better deposition uniformity. This can be ascribed to the higher pigment dosage at the higher spray volumes. In our trials, pigment concentration was not increased with reduction in water volumes (e.g. 1× at high volume *vs.* 2× at low volume). Thus, less pigment was deposited per canopy area at lower spray volumes. However, normalised deposition values indicated that sprays were more efficient at the lower spray volumes. Deposition efficiency values indicated some variation in adjuvant effects (*i.e.* better or poorer efficiency at lower spray volumes) relative to that observed in the control treatment. This can can quite simply be attributed the adjuvant concentration × spray volume interaction, as was demonstrated by van Zyl *et al.* (2010b). Previous studies (Gaskin *et al.*, 2004; van Zyl *et al.*, 2014) demonstrated that organocilicone adjuvants were more efficient when used

at lower spray volumes. Our findings from trial 1 consurs with this observation, but in trial 2 and particularly in trial 3, Break-Thru S240 and ceratin other adjuvants performed better at the higher spray volumes. This might again be an artefact of the faster tractor speed used for lower spray volumes and needs to be investigated further.

Deposition quantity values generally exceeded the FPC benchmarks in the high volume applications. Lower volume applications generally did not exceed FPC benchmarks, indicating potentially poorer control at the 1x dosage applied. Future studies should keep amend tracer dosage per canopy area constant throughout evaluation so that more accurate volume comparisons can be made and to evaluate the accuracy of normalised spray deposition efficiency values.

Detrimental effects of adjuvants on deposition parameters (most commonly on deposition quantity) can mostly be ascribed to run-off induced by the high spray volumes and spreading action of adjuvant treatments; this was exacerbated by adjuvants with a higher degree of spreading action. Adjuvants used in this study was classified wetters/spreaders (Break-Thru S240, Entreé, Exit, Wetcit, Villa51, Citrole100 and Herbiplus), penetrants (Break-Thru S240, Citrole100, Herbiplus) and stickers (Nu-Film-17). Adjuvants can improve deposition by improving/changing deposition factors on the target surface (relieving hydrophobic tension between the droplet and the target surface and therefore improving deposition) (Holloway *et al*., 2000), as well as through improved droplet formation as a result of its modification of the spray liquid (Butler Ellis *et al*., 1997; Butler Ellis and Tuck, 1999; Spanoghe *et al*., 2007) and through improving/changing deposition factors on the target surface (relieving hydrophobic tension between the droplet and the target surface and therefore improving deposition) (Holloway *et al*., 2000). When used at high application volumes, the wetting and spreading of droplets, followed by droplet coalescing and then run-off can be aggravated (Faers and Pontzen, 2008, van Zyl *et al*., 2010a). Therefore, for adjuvants to contribute to the amount of product landed and retained (deposition quantity) there must be as little product loss due to run-off, blow-off and drift as possible. Slower tractor speeds might also have contributed to a higher degree of blow-off. In trial 3, tractor speeds were generally higher (2.5 to 4.8 km h^{-1}) and the adjuvant benefits were more notable than in trials 1 and 2 where generally slower tractor speeds were used (1.6 to 3.4 km h^{-1}). Whitney *et al.* (1989) and Salyani and Whitney (1990) found that increasing sprayer speeds did not necessarily reduce deposition.

Higher spray volumes past the point of run-off generally lead to more run-off. Cunningham and Harden (1998) determined that the amount of spray retained by citrus trees rapidly decreases above 2000 L ha^{-1}. However, in trial 3, higher spray volumes were used (10389 and 5411 L ha^{-1})

but higher deposition quantity was achieved and adjuvants generally improved deposition. The trees sprayed in this trial was much larger than the trees in other trials, thus having a higher spray volume retaining ability (higher point of run-off). Other reasons for this anomaly, might be higher tractor speed (as mentioned previously) as well as the specific sprayer used in the trial, which might have influenced droplet spectra and transport to the target.

Few studies have compared spray deposition effects of adjuvant products or active ingredients on fruit trees. Although there were no best performer from this study it was evident that certain adjuvants performed better and more consistently, notably the organosilicone surfactant (Break-Thru S240) and the terpine oil/resins sticker (Nu-Film-17), followed by the mineral oils (Citrole100 and Herbiplus). The emulsifiable vegetable-based oils (Entrée and Exit), alkylpolyethene glycor ether (Villa51) and inorganic compound (Wetcit) realised more inconsistent deposition parameters. This concurred with conclusions following a laboratory study on difficult-to-wet field bean, pea and barley foliage done by Holloway *et al.* (2000). Holloway *et al.* (2000) also stated that the amount (dose rate) of the adjuvant used will have an effect on the deposition parameters obtained as was the case with Exit (vegetable oil), which worsened deposition quantity and quality significantly; the high prescribed concentration used reduced droplet surface tension too much and led to excessive run-off. Gaskin *et al*. (2004) highlighted the importance of using the correct adjuvant concentration together with the correct spray volume. Entrée, the same product as Exit, only used at a lower concentration, performed markedly better in terms of deposition quantity and uniformity, supporting previous findings above of using adjuvants at the correct concentration.

Suboptimal performance of adjuvants in our trials can also be ascribed to the surface characteristics of citrus leaves being smooth and moderately wettable in relation to other plant species surfaces (Neinhuis and Barthlott, 1997). The size, speed and projection angle of the droplet to the target and surface characteristics of the target has a major effect on droplet impact (Boukhalfa *et al*., 2014; Zwertvaegher *et al*., 2014; Mayo *et al*., 2015; Massinon *et al*., 2017). As indicated by previous authors (Holloway *et al*., 2000; Xu *et al*., 2011; Dorr *et al*., 2015; Mayo *et al*., 2015), adjuvants influence droplet formation, impaction followed by deflection, bounce, shattering and adhesion/retention. Dorr *et al.* (2015) and Massinon *et al.* (2017) found that on easy to moderately wettable and smooth surfaces (e.g. avocado leaves) the primary outcome is droplet adhesion whilst reflection, bounce and shattering was low due to the less complex leaf surface. The latter effects were reduced with the addition of a trisiloxane ethoxylate (similar to Break-Thru S240) and a vegetable oil-based adjuvant (similar to Exit, Entreé and Villa51). The more complex a target surface (leaves with hairs, trichomes, stomata, rough texture, protuberant

veins and thick and complex wax layers), the harder it is to overcome the physical and chemical interactions between the droplet and the leaf surface (Smith *et al*., 2000; Dorr *et al*., 2015). Citrus leaves can be regarded as moderately wettable with a relatively smooth cuticular surface and well developed wax layer (Baker *et al*., 1975). Together with the high spray volumes used, adjuvants might have increased run-off, which might explain suboptimal performance of adjuvants in our study. It is the author's opinion that the addition of adjuvants to PPP sprays on hard to wet/complex leaf surfaces might have much more beneficial effect than what was found on citrus leaves. However, this needs to be evaluated.

In conclusion, the addition of certain adjuvants can have beneficial effects on spray deposition parameters on citrus foliage. However, adjuvant concentration, spray volume and canopy characteristics (and possibly other factors, such as sprayer dynamics and spray speed) significantly influenced these effects. Furthermore, adjuvant addition to surface active fungicides (systemic, meso-systemic and translaminar), fungicides formulated with adjuvants might further influence deposition parameters. Inert contact fungicide addition in previous studies did not influence deposition parameters (Rossouw *et al*., 2018). Deposition efficiency results indicated that lower volume sprays with adjuvants were more efficient, especially with Break-Thru S240 and Nu-Film-17. Certain adjuvants performed better and more consistently than others, highlighting the complexity of factors that might influence spray deposition. In certain cases, deposition parameters were adversely affected, and use of adjuvants at too high concentrations should be avoided, particularly in high volume applications.

REFERENCES

Baker, A.B., Procopiou, J., Hunt, M.H., 1975. The cuticles of Citrus species. Composition of leaf and fruit waxes. Journal of the Science of Food and Agriculture 26: 1093-1101.

Bonnet, D.G., 2006. Confidence interval for a coefficient of quartile variation. Computational Statistics and Data Analysis 50: 2953-2957.

Boukhalfa, H.H., Massinon, M., Belhamra, M., Lebeau, F. 2014. Contribution of spray droplet pinning fragmentation to canopy retention. Crop Protection 56: 91-97.

Butler Ellis, M.C. and Tuck, C.R. 1999. How adjuvant influence spray formation with different hydraulic nozzles. Crop Protection 18: 101-109.

Butler Ellis, M.C., Tuck, C.R., Miller, P.H.C. 1997. The effect of some adjuvants on sprays produced by agricultural flat fan nozzles. Crop Protection 16: 41-50.

Brink, J.C., Holz, G., Calitz, F.J., Fourie, P.H., 2004. Development of a protocol to quantify spray deposits on grape bunches. 7th International Symposium on Adjuvants for Agrochemicals (*ISAA 2004*), South Africa: 230-236.

Brink, J.C., Holz, G., Fourie, P.H., 2006. Effect of fungicide spray cover on *Botrytis cinerea* infection in grape bunches. South African Journal for Enology and Viticulture 27: 51-56.

CBS Expert Panel, 2013. Response to EFSA panel on plant health, 2013-draft Scientific opinion on the risk of *Phyllosticta citricarp*a (*Guignardia citricarpa*) for EU territory with identification and evaluation of risk reduction options. http://www.citrusres.com/market/access.

CBS Expert Panel, 2014. Comments on the European Union Food Safety Authority's Pest Risk Assessment for *Phyllosticta citricarpa*. http://www.citrusres.com/market-access.

CBS Expert Panel, 2015. Response to the EFSA 2015 statement on comments made by an international panel of scientists regarding EFSA's 2014 risk assessment for *Phyllosticta citricarpa*. http://www.citrusres.com/market-access.

Cunha, J.P., Chueca, P., Garcerá, C., Moltó, E. 2012. Risk assessment of pesticide spray drift from citrus applications with air-blast sprayers in Spain. Crop Protection 42: 116-123.

Cunningham, G.P., Harden, J. 1998. Air-tower sprayers increase application efficiency in mature citrus trees. Australian Journal of Experimental Agriculture 38: 871-877.

Cunningham, G.P., Harden, J. 1999. Sprayers to reduce spray volumes in mature citrus trees. Crop Protection 18: 275-281.

Dekeyser, D., Foqúe, D., Duga, A.T., Verboven, P., Hendrickx, N., Nuyttens, D. 2014. Spray deposition assessment using different application techniques in artificial orchard trees. Crop Protection: 64: 187-197.

Dorr, G.J., Wang, S., Mayo, L.C., McCue, S.W., Forster, W.A., Hanan, J., He, X. 2015. Impaction of spray droplets on leaves: influence of formulation and leaf character on shatter, bounce and adhesion. Experiments in Fluids 56:1-17.

Duga, A.T., Ruysen, K., Dekeyser, D., Nuyttens, D., Bylemans, D., Nicolai, B.M., Verboven, P. 2015. Spray deposition profiles in pome fruit trees: Effects of sprayer design, training system and tree canopy characteristics. Crop Protection 67: 200-213.

EFSA, 2014. Scientific opinion on the risk of *Phyllosticita citricarpa* (*Guignardia citricarpa*) for the EU territory with identification and evaluation of risk reduction options. European Food Saftey Journal 12.

EPPO,. 2014. 2014 PQR - EPPO database on quarantine pests (available online). http://www.eppo.int.

Faers, M.A., Pntzen, R. 2008. Factors influencing the association between active ingredient and adjuvant in the leaf deposit of adjuvant-containing suspoemulsion formulations. Pest Management Science 64: 820-833.

Farooq, M., Salyani, M. 2002. Spray penetration into citrus tree canopy from two air-carrier sprayers. ASAE Meeting Presentation 02-1038: 1-12.

Forster, W.A., Mercer, G.N., Schou, W.C. 2012. Spray droplet impaction models and their use within AGDISP software to predict retention. New Zealand Plant Protection 65: 85-92.

Fourie, P.H., du Preez., M., Brink, J.C., Schutte, G.C. 2009. The effect of run-off on spray deposition and control of Alternaria brown spot of mandarins. Australasian Plant Pathology 38: 173-182.

Garcerá, C., Moltó, E., Chueca, P. 2014. Factors influencing the efficacy of two organophosphate insecticides in controlling California red scale, *Aonidiella aurantia* (Maskell). A basis for reducing spray application volume in Mediterranean conditions. Pesticide Management Science 70: 28-38.

Garcerá, C., Fonte, A., Moltó, E., Chueca, P. 2017. Sustainable use of pesticide applications in citrus: A support tool for volume rate adjustment. International Journal of Environmental Research and Public Health 14: 1-14.

Gaskin, R.E., Elliott, G.S., Manktelow, D.W.L., Skinner, S.J. 2004. Use of a superspreader adjuvant to reduce spray application volumes on avocados. New Zealand Plant Protection 57: 266-270.

Gaskin, R.E., Manktelow, D.W.L., Steele, K.D. 2006. Adjuvant and application technologies to minimise off-target drift from kiwifruit sprays. New Zealand Crop Protection 59: 217-222.

Gaskin, R.E., Steele, K.D. 2009. A comparison of sticker adjuvants for their effects on retention and rainfastening of fungicide sprays. New Zealand Crop Protection 62: 339-342.

Gent, D.H., Schwartz, H.F., Nissen, S.J. 2003. Effect of commercial adjuvants on vegetable crop fungicide coverage, absorbtion and efficacy. Plant Disease 87: 591-597.

Green, J.M., Beestman, G.B. 2007. Recently patented and commercialized formulation and adjuvant technology. Crop Protection 26: 320-327.

Grout, T.G., 1997. Spray volumes and coverage requirements for citrus in South Africa. Citrus J. 31: 19-20.

Grout, T.G., 2003. Use of plant protection products. Section 2.7. In: Grout, T.G. (Ed.), Citrus Production Guidelines. Integrated Pest Management, vol. III. Citrus Research International, Nelspruit, South Africa.

Hazen, J.L., 2000. Adjuvants-Terminology, classification, and chemistry. Weed Technology 14: 773-784.

Holloway, P.J., 1994. Physicochemical factors influencing the adjuvant-enhanced spray deposition and coverage of foliage-applied agrochemicals. Pages 83-106 in: Holloway, P.J., Rees, R.T., Stock, D. Interactions between Adjuvants, Agrochemicals and Target Organisms. Springer-Verlag, Berlin.

Holloway, P.J., Butler Ellis, M.C., Webb, D.A., Western, N.M., Tuck, C.R., Hayes, A.L. and Miller, P.C.H. 2000. Effects of some agricultural tank-mix adjuvants on the deposition efficiency of aqueous sprays on foliage. Crop Protection 19: 27-37.

Khot, R.L., Ehsani, R., Albrigo, G., Larbi, A.P., Landers, A., Campoy, C., Wellington, C. 2012., Air-assisted sprayer adapted for precision horticulture: Spray patterns and deposition assessments in small-sized citrus canopies. Biosystems Engineering 113: 76-85.

Knoche, M., Noga, G., Lenz, F. 1992. Surfactant induced phytotoxicity: evidence for interaction with epicuticular wax fine structure. Crop Protection 11: 51-56.

Kotzé, J.M., 1981. Epidemiology and control of citrus black spot in South Africa. Plant Disease 65: 945-950.

Kotzé, J.M., 2000. Citrus black spot. Pages 23-25 in: Compendium of Citrus Diseases (second Edition). Timmer, L.W., Garnsey, S.M. and Graham, J.H. APS Press, The American Phytopathological Society, St. Paul Minnesota, USA.

Massinon, M., De Cock, N., Forster, A.W., Nairn, J.J., McCue, S.W., Zabkiewics, J.A., Lebeau, F. 2017. Spray droplet impaction outcomes for different plant species and spray formulations. Crop Protection 99: 65-75.

Mayo, L.C., McCue, S.W., Moroney, T.J., Forster, W.A., Kempthorne, D.M., Belward, J.A., Turner, I.W. 2015. Simulating droplet motion on virtual leaf surfaces. Royal Society of Open Science 2: 140528

Miller, P.C.H., Butler Ellis, M.C. 1997. A review of spray generation, delivery to the target and how adjuvants influence the process. Plant Protection Quarterly 12:33-38.

Neinhuis, C., Barthlott, W. 1997. Characterization and distribution of water-repellent, Self-cleaning plant surfaces. Annals of Botany 79: 667-677.

Rossouw, C.J., Fourie, P.H., van Zyl., J.G., Hoffman, J.E., McLeod, A. 2018. Rainfastness of mancozeb on apple seedlings determined through deposition quantification of mancozeb residue and fluorescent pigment. Crop Protection 106: 93-102.

Salyani, M. 2015. Florida citrus pest management guide: Pesticide application technology. Institute of Food and Agricultural Sciences, University of Florida, Gainesville, United States of America. http://edis.ifas.ufl.edu.

Salyani, M., Whitney, J.D., 1990. Ground speed effect on spray deposition inside citrus trees. Transactions of the American Society of Agricultural and Biological Engineers 33: 361-366.

Salyani, M., Farooq, M., 2005. Drift potential of citrus air-carrier sprayers. Proceedings of the Florida State Horticultural Society 117: 130-135.

Salyani, M., Farooq, M., Sweeb, R.D. 2007. Spray deposition and mass balance in citrus orchard applications. Transactions of the American Society of Agricultural and Biological Engineers 50: 1963-1969.

Salyani, M., Miller, D.R., Farooq, M., Sweeb, R.D. 2013. Effects of sprayer operating parameters on airborne drift from citrus air-carrier sprayers. Agricultural Engineering International: CIGR Journal 15: 27-36.

Schutte, C.G., 1996. First report of Alternaria brown spot on Star Ruby grapefruit in South Africa. Citrus Journal 6: 24.

Schutte, G.C., Beeton, K.V., Kotzé, J.M., 1997. Rind stippling on Valencia oranges by copper fungicides used for control of citrus black spot in South Africa. Plant Disease 81: 851-854.

Schutte, G.C., Kotze, C., van Zyl, J.G., Fourie, P.H., 2012. Assessment of retention and persistence of copper fungicides on orange fruit and leaves using fluorometry and copper residue analyses. Crop Protection 42: 1-9.

Smith, D.B., Askew, S.D., Morris, W.H., Shaw, D.R., Boyette, M. 2000. Droplet size and leaf morphology effects on pesticide spray deposition. Transactions of the American Society of Agricultural Engineers 43: 255-259.

Spanoghe, P., De Schampheleire, M., Van der Meeren, P., Steurbaut, W., 2007. Influence of agricultural adjuvants on droplet spectra. Pest Management Science 63: 4-16.

Steurbaut, W. 1993. Adjuvants for use with foliar fungicides. Pesticide Science 38: 85-91.

Stover, E., Salvatore, J. 2002. Influence of spray volume, ground speed and a sensor-actuated spray controller on melanose control and fruit marking in fresh Indian river grapefruit. Proceedings of the Florida State Horticultural Society 115: 1-5.

Van Zyl, J.G., Fourie, P.H., Schutte, C.G., 2013. Spray deposition assessment and benchmarks for control of Alternaria brown spot on mandarin leaves with copper oxychloride. Crop Protection 46: 80-87.

Van Zyl, J.G., Sieverding, E.G., Viljoen, D.J., Fourie, P.H. 2014. Evaluation of two organosilicone adjuvants at reduced foliar spray volumes in South African citrus orchards of different canopy densities. Crop Protection 64: 198-206.

van Zyl, S.A., Brink, J.C., Calitz, F.J., Coertze, S., Fourie, P.H., 2010(a). The use of adjuvants to improve spray deposition and *Botrytis cinerea* control on Chardonnay grapevine leaves. Crop Protection 29: 58-67.

van Zyl, S.A., Brink, J.C., Calitz, F.J., Fourie, P.H., 2010(b). Effects of adjuvants on deposition efficiency of fenhexamid sprays to Chardonnay grapevine foliage. Crop Protection 29: 843-852.

Walklate, P.J., Cross., J.V., Richardson, G.M., Baker, D.E. 2006. Optimising the adjustment of label-recommended dose rate for orchard spraying. Crop Protection 25: 1080-1086.

Walklate, P.J., Cross, J.V. 2012. An examination of Leaf-Wall-Area dose expression. 35: 132-134.

Whitney, J.D., Salyani, M., Churchill, D.B., Knapp, J.L., Whiteside, J.O., Littell, R.C. 1989. A field investigation to examine the effects of sprayer type, ground speed, and volume rate on spray deposition in Florida citrus. Journal of Agricultural Engineering Research 42: 275-283.

Xu, L., Zhu, H., Ozkan, H.E., Bagley, W.E., Krause, R.C. 2011. Droplet evaporation and spread on hairly leaves associated with type and concetration of adjuvants. Pesticide Management Science 67: 842-851.

Zwertvaegher, I.K., Verhaeghe, M., Brusselman, E., Verboven, P., Lebeau, F., Massinon, M., Nicolaï, B.M., Nuyttens, D. 2014. The impact and retention of spray droplets on a horizontal hydrophobic surface. Biosystems Engineering 126: 82-91.

Table 1. Properties of adjuvants evaluated.

Trade name	Registration holder	Main components	Properties	Chemistry classification	Active ingredient (g L^{-1})	Registered concentration (mL hL^{-1})
Break-Thru S240	Evonik Degussa Africa	Polyether-polymethylsiloxane-copolymer	Non-ionic-penetrant-surfactant	Organosilicone	1000	3
Citrole100	Total SA	Mineral oil	Mineral oil spreader/insecticide	Paraffinic oil complex	790	300
Entreé	Miller Chemicals SA	Emulsifiable vegetable oil	Non-ionic-wetter-spreader	Vegetable oil complex	818	60
Exit	Miller Chemicals SA	Emulsifiable vegetable oil	Non-ionic-wetter-spreader	Vegetable oil complex	819	250
Herbiplus	Total SA	Mineral oil	Mineral oil spreader/pH buffer	Paraffinic oil complex		300
Nu-Film-17	Miller Chemicals SA	Di-1-p-menthene	Non-ionic-sticker-spreader	Terpene oil	905	50
Villa51	Villa Crop Protection	Isotridecanol	Wetter-spreader	Alkylpolyethene glycol ether	918	18
Wetcit	Oro Agri SA	Borax and orange oil	Wetter-spreader-penetrant	Inorganic compound	10 and 50	100

Table 2. Deposition quantity realised by the water only control treatment (control) and adjuvant treatments on outer and inner canopy leaves following sprays at 4232 L ha^{-1} in 'Eureka' lemon and 'Star Ruby' grapefruit orchards.

Treatment[a]	Deposition quantity (FPC%)		
	Outer canopy[b]	Inner canopy[b]	Outer:Inner (%)[c]
Control	2.94 b	1.27 ef	43
Break-Thru S240	3.84 a	2.19 cd	57
Nu-Film-17	3.72 a	2.19 cd	59
Villa51	2.70 bc	1.33 ef	49
Herbiplus	2.76 b	1.55 e	56
Wetcit	2.22 cd	1.47 e	66
Exit	1.68 de	0.84 f	50

[a] Refer to Table 1 for recommended adjuvant concentrations.

[b] Values followed by the same letter do not differ significantly ($P > 0.05$) according to Student T least significance difference test.

[c] Ratio of deposition quantity between outer and inner canopy leaves, calculated as mean FPC% on inner canopy leaves / mean FPC% on outer canopy leaves × 100.

Table 3. Mean deposition uniformity and quality realized by the water only control treatment (control) and adjuvant treatments following sprays at 4232 L ha^{-1} in 'Eureka' lemon and 'Star Ruby' grapefruit orchards.

Treatment[a]	Deposition uniformity (CV%)[b]	Deposition quality (ICD%)[b]
Control	73.72 a	52.23 cd
Break-Thru S240	66.37 ab	46.14 e
Nu-Film-17	62.92 b	47.73 de
Villa51	65.34 ab	55.17 bc
Herbiplus	62.18 b	53.53 bc
Wetcit	62.13 b	59.07 b
Exit	75.10 a	64.89 a

[a] Refer to Table 1 for recommended adjuvant concentrations.

[b] For each parameter separately, values followed by the same letter do not differ significantly ($P > 0.05$) according to Student T least significance difference test.

Table 4. Mean deposition quantity realised by the water only control treatment (control) and adjuvant treatments on leaves following sprays at high (6850 to 7535 L ha^{-1}) and medium (3430 to 3768 L ha^{-1}) spray volumes in a 'Bahianina' navel orchard in two separate trials.

Treatment[a]	Deposition quantity (FPC%)[b]					
	High volume			Medium volume		
	Outer canopy	Inner canopy	Outer:Inner (%)[c]	Outer canopy	Inner canopy	Outer:Inner (%)[c]
Control	3.91 a	3.12 bc	80	2.57 c-f	2.26 d-g	88
Break-Thru S240	3.34 ab	3.26 b	98	2.03 e-k	1.47 i-m	73
Nu-Film-17	3.06 bc	2.76 bcd	90	2.04 e-j	1.46 j-m	72
Villa51	2.60 cde	2.26 d-g	87	2.09 e-i	1.60 h-m	77
Citrole100	2.75 bcd	2.58 c-f	94	2.06 e-j	1.49 h-m	72
Wetcit	2.62 cde	2.28 d-g	87	2.10 e-h	1.77 g-l	84
Entreé	2.39 d-g	1.96 f-k	82	1.06 nm	0.44 n	42
Exit	1.88 g-k	1.61 h-m	86	1.42 klm	1.18 lm	83

[a] Refer to Table 1 for recommended adjuvant concentrations.

[b] Deposition quantity values followed by the same letter do not differ significantly ($P > 0.05$) according to Student T least significance difference test.

[c] Ratio of deposition quantity between outer and inner canopy leaves, calculated as mean FPC% on inner canopy leaves / mean FPC% on outer canopy leaves × 100.

Table 5. Mean deposition quantity realised by the water only control treatment (control) and adjuvant treatments on inner and outer canopy leaves following sprays at 10389 and 5114 L ha^{-1} on a 'Cara Cara' navel orchard.

Treatment[a]	Deposition quantity (FPC%)					
	10389 L ha^{-1}			5411 L ha^{-1}		
	Outer canopy[b]	Inner canopy[b]	Outer:Inner (%)[c]	Outer canopy[b]	Inner canopy[b]	Outer:Inner (%)[c]
Control	4.90 cd	2.86 ij	58	3.00 hi	1.37 lm	46
Break-Thru S240	6.30 b	3.19 ghi	51	4.45 de	1.85 klm	42
Nu-Film-17	8.13 a	3.88 efg	48	5.28 c	2.09 jkl	40
Villa51	4.93 cd	2.88 hij	58	3.66 fgh	1.63 klm	45
Citrole100	5.40 c	3.32 f-i	61	3.84 efg	1.21 m	32
Wetcit	5.35 c	3.94 efg	74	4.03 ef	2.18 jk	54
Entreé	6.32 b	3.35 f-i	53	4.05 ef	1.07 m	26

[a] Refer to Table 1 for recommended adjuvant concentrations.

[b] Values followed by the same letter do not differ significantly ($P > 0.05$) according to Student T least significant difference test.

[c] Ratio of deposition quantity between outer and inner canopy leaves, calculated as mean FPC% on inner canopy leaves / mean FPC% on outer canopy leaves × 100.

Table 6. Mean deposition uniformity realised by the water only control treatment (control) and adjuvant treatments following sprays at 10389 and 5114 L ha^{-1} on a 'Cara Cara' navel orchard.

Treatment[a]	Deposition uniformity (CV% between leaves)[b]	
	10389 L ha^{-1}	**5411 L ha^{-1}**
Control	68.19 a-d	69.24 abc
Break-Thru S240	56.91 efg	71.48 ab
Nu-Film-17	56.76 efg	71.46 ab
Villa51	58.57 d-g	61.16 c-f
Citrolle 100	52.80 fg	65.56 b-e
Wetcit	56.73 efg	71.27 abc
Entreé	50.05 g	76.62 a

[a] Refer to Table 1 for recommended adjuvant concentrations.

[b] Values followed by the same letter do not differ significantly ($P > 0.05$) according to Student T least significant difference test.

CHAPTER 5

Influence of adjuvants on spray deposition and control of Alternaria brown spot on mandarin leaves following sprays with copper oxychloride

ABSTRACT

The effects of adjuvants on deposition quantity, quality and biological efficacy of copper oxychloride against Alternaria brown spot (ABS) on mandarin leaves were determined. Detached young 'Nova' mandarin leaves were sprayed at pre- (1 mL) and post- (3 mL) run-off volumes with copper oxychloride, a yellow fluorescent pigment and selected adjuvants at recommended concentrations. Deposition quantity and quality were assessed using fluorometry, photomacrography and digital image analysis. Post-run-off sprays were repeated to simulate orchard spray deposition parameters. Leaves were subsequently spray-inoculated with a spore suspension of *Alternaria alternata* (causal agent of ABS), moist-incubated for *c.* 48 h and symptom expression rated by means of image analysis. Differences in deposition patterns could visually be observed between pre- and post-run-off volumes and adjuvant types. At pre-run-off volumes very small to larger droplet remnants were deposited, uniformly distributed on the leaf surface, whereas post-run-off application showed non-uniform wave-like and streaking deposition patterns. Adjuvant treatments varied significantly in deposition quantity (fluorescent particle coverage %; FPC%) and quality (ICD% and protected leaf area %) and disease control achieved. Higher deposition quantity, beter quality and higher Cu residues was realized at pre- *vs.* post-run-off volumes. Adjuvants did not improve deposition parameters compared with the control treatment at both spray volumes. In both trials, Break-Thru S240, Nu-Film-17 and Citrole100 improved Cu residue with post-run-off sprays. Leaf infection analysis indicated that copper oxychloride sprays with Entreé achieved markedly better control (73.14%) than copper oxychloride alone (54.82%), but this and the other adjuvant treatments (64.19 to 52.65%) did not differ significantly from the control treatment. Predicted ABS control based on deposition quantity was fairly accurate (r = 0.647); however, only Entreé had a strong linear relationship (r = 0.711), and the results indicate that deposition quantity and Cu-residues alone could only partially explain the level of control achieved. The effects of deposition quality and other direct or indirect effects of adjuvant on ABS control should be studied to elucidate these findings.

INTRODUCTION

Contact copper fungicides and bactericides form an integral part of fruit and foliar disease control in citrus production all over the world. It is an economical choice of fungicide, has an effective protectant activity, is important for resistance management and in some countries, is the only registered active for chemical control of certain diseases. It is used for the control of most citrus fruit and foliar diseases, including Alternaria brown spot (ABS; *Alternaria alternata*

(Fr: Fr) Keissl.) (Solel *et al*., 1997; Swart *et al*., 1998; Miles *et al*., 2005; Mondal *et al*., 2007; Vicent *et al*., 2007; 2009; Dewdney and Timmer, 2012), melanose (Timmer and Zitko, 1996; Timmer *et al*., 1998; Mondal *et al*., 2007; Dewdney and Timmer, 2012), citrus canker (Mondal *et al*., 2007; Behlau *et al*., 2008; 2010; Dewdney and Graham, 2012), greasy spot (Timmer and Zitko, 1996; Dewdney and Timmer, 2012), citrus black spot (CBS) (Kotzé, 1981; 2000; Schutte *et al*., 1997; Dewdney *et al*., 2012), citrus scab (Mondal *et al*., 2007; Dewdney and Timmer, 2012) and is in some cases the only available and/or viable alternative where dithiocarbamates cannot be used.

For protectant copper fungicide sprays to be effective, it has to be present on the target surface before fungal or bacterial inoculum, especially during critical infection periods since curative applications are usually ineffective. Effective deposition of the active ingredient on the target surface (citrus leaves, twigs or fruit) is needed for effective disease control (Holownicki *et al*., 2002), especially since copper do not redistribute when citrus fruit and foliage grow and as it is prone to weathering (Vicent *et al*., 2007; Schutte *et al*., 2012).

Citrus trees are often large and dense. This complicates adequate deposition on difficult-to-reach inner canopy leaves and fruit (van Zyl *et al*., 2014; Chapter 4). Hence, fruit and foliar diseases are currently being controlled by regular fungicide spray applications consisting of spray volumes ranging from 6,000 to 10,000 L ha^{-1} (medium to full cover sprays, respectively) in citrus producing areas of South Africa. These methods of application provide an acceptable balance between efficacy and efficiency based on existing economic conditions (Grout, 1997; 2003) and serves as a safety buffer for calibration and application errors. Reduced volume application at adjusted label-recommended dose rates has the potential to prevent significant product loss, negative environmental impact and to realise more time-efficient spray application in citrus (Cunningham and Harden, 1999; van Zyl *et al*., 2014) and other orchard crops (Baldoin *et al*., 2001). However, as near-perfect control of phytosanitary pests and diseases is required to ensure market access, spray application and therefore pest and disease management must consider implementation and optimisation of all relevant inputs and parameters.

Spray application is a complex procedure due to the large number of contributing factors influencing spray deposition (Dekeyser *et al*., 2014). Major influences on spray deposition efficiency and efficacy include the target surface characteristics (be it leaves, twigs, fruit), the complexity and wettability of the target (e.g. leaf surface characteristics: hairs, trichomes, stomata, veins, and the wax layer) (Dorr *et al.*, 2015), canopy geometry and density (Hall *et al*., 1991; Jejčič *et al*., 2011; van Zyl *et al*., 2014; Duga *et al*., 2015), prevailing environmental conditions (Salyani, 2005; 2006), the use of appropriate equipment (Cunningham and Harden, 1998a; 1998b; 1999; Salyani, 2005; 2006; Furness *et al*., 2006b; Duga *et al*., 2015; Chapter 4), spray technique (Salyani and Whitney, 1990; Furness *et al*., 1998; Salyani and Farooq, 2004), spray volume (Salyani and Hoffmann, 1996; Cunningham and Harden, 1999; Stover *et*

al., 2002b; Fourie *et al*., 2009; van Zyl *et al*., 2014), the fungicide or pesticide used (Sundaram and Sundaram, 1987; Zabkiewicz, 2007), the influence of adjuvants (Butler Ellis *et al*., 1997; Gent *et al*., 2003; Green and Beestman, 2007; Spanoghe *et al*., 2007; van Zyl *et al*., 2010a; 2010b; van Zyl *et al*., 2014; Chapter 4), droplet formation, impaction, deposition (Spanoghe *et al*., 2007; Xu *et al*., 2011; Mayo *et al*., 2015; Dorr *et al*., 2015) and the complex interaction between these factors (Whitney *et al*., 1988; 1989; Salyani, 1994; 2005; 2006; Stover *et al*., 2002a; Grout, 2003; van Zyl el al., 2014; Chapter 4).

Adjuvants can provide citrus growers with a powerful tool to optimise spray application through improved spray deposition of the active ingredient on the target surface (de Ruiter *et al.*, 1990; Holloway *et al*., 2000; Gent *et al*., 2003; van Zyl *et al*., 2014; Chapter 4), if used correctly. Adjuvants added to spray mixtures influence the surface tension of spray droplets at the air-liquid interface and on the contact angle of the liquid-plant interface, mostly by lowering both. Thus, droplets are less prone to shattering, deflection and bouncing on impact with the leaf surface, reducing off-target losses and improving deposition, especially on hard to wet (hydrophobic) targets (Dorr *et al*., 2015; Mayo *et al*., 2015). Adjuvants are used with most fungicide spray application in South African citrus orchards, yet little data and literature exist on the effects of different adjuvant types, such as wetting, spreading, sticking and penetration agents, on fungicide deposition, as well as the influence on biological efficacy, especially with current application methodology.

Research on grapevine (van Zyl *et al*., 2010a; 2010b) has shown the potential of adjuvants to improve deposition quantity and quality, as well as disease control. However, spray applications using the extremes of recommended concentrations of certain adjuvants, or set concentrations at different spray volumes, realised significantly different results (van Zyl *et al*., 2010b), indicating the need for more specific recommendations for each crop and application. On avocados (Gaskin *et al*., 2004; 2008) and kiwis (Gaskin *et al*., 2006), the ability of adjuvants to reduce spray volumes and off-target drift has been demonstrated. The ability of adjuvants to improve retention (rain-fastness) of fungicide sprays with sticking agents on cabbage and bean has also been shown (Gaskin and Steele, 2009). However, contrary results were reported by Rossouw *et al.* (in press) who found that Nu-Film-P, a sticker-spreader adjuvant, did not improve rainfastness of mancozeb on apple leaves. Van Zyl *et al*. (2014) demonstrated that the effective use of adjuvants in citrus orchards has the potential to improve deposition parameters at reduced spray volumes. Deposition quality and uniformity on leaves with two organosilicone formulations were improved at lower application volumes than the norm; however, these benefits were not as evident in very dense canopies, illustrating the importance of canopy management when spraying at reduced volumes. In Chapter 4, the potential benefit of adjuvants on deposition parameters, most notably on quality and uniformity, was also shown, when used at the correct product concentration for the application. The study indicated the detrimental effect adjuvants can have on deposition parameters, especially quantity, if used

at post run-off spray volumes; run-off effects were exacerbated when the adjuvant was used at too high concentrations.

Van Zyl *et al.* (2013) developed deposition quantity benchmarks to predict the effect of spray retention on control of ABS. Adjuvants affect deposition quantity as well as quality and the extent to which these combined adjuvant effects influenced biological efficacy of a fungicide spray was not known.

The objective of this laboratory study was to evaluate the effects of commonly used adjuvants in spray mixtures with copper oxychloride on deposition quantity and quality parameters at pre- and post-run-off volumes. To simulate post-run-off deposition achieved in orchard evaluations and what is commonly achieved in citrus spray application in South Africa, the influence of post-run-off spray mixtures on the control of ABS were also evaluated. A previously developed deposition assessment protocol and efficacy benchmark model (van Zyl *et al.*, 2013, 2014) was used in this study. As motivated in van Zyl *et al.* (2013), the model pathosystem chosen for this study was ABS on mandarin leaves given its similarities to other important citrus fruit and foliar diseases and relative ease of use.

MATERIALS AND METHODS

Evaluation of pre- and post-run-off spray application

Leaves

ABS-susceptible 'Nova' mandarin (*C. reticulata* Blanco; hybrid of Clementine 'Fina' and tangelo 'Orlando') trees were grown in 10-L plastic pots in a glasshouse at 27°C. Drip irrigation and a monthly application of slow release fertiliser (3:1:2 of N:P:K) were used to maintain the plants. Trees were regularly pruned to stimulate young growth (flush) production for use in experiments and too keep the trees small.

Spray application

Twenty to 30 young (flush) shoots were cut from 'Nova' mandarin trees in the glasshouse. Upper leaf surfaces of untreated, fresh detached young leaves (smallest: 2 to 3 days old, ±15×8 mm; largest: 7 to 10 days old, ±55×30 mm) were sprayed by means of a gravity feed mist spray gun (ITW DEVILBISS Spray Equipment Products, USA) with a fluid nozzle tip of 1.5 mm in diameter mounted on a spray frame (steel framework 800×1410×660 mm). For each treatment, eight leaves were arranged by size, smallest to largest, per treatment to provide a similar total leaf area sprayed per individual leaf and between treatments. A single leaf was positioned on a wire mesh tray (angled at 30° to the bench top), while the spray gun was mounted at a distance of 600 mm away aiming squarely at the target. A pre- (1 mL) and post-run-off (3 mL) spray volume of copper oxychloride [Villa Copper Oxychloride, 85% WP (Villa Crop Protection SA, Kempton Park, South Africa); copper oxychloride with 50% metallic copper equivalent; 2 g L^{-1}], yellow fluorescent pigment [SARDI Yellow Fluorescent Pigment

40% EC (SARDI, Loxton, South Australia); 1 mL L^{-1}] and deionised water were sprayed alone or together with spray adjuvants at recommended concentrations (Table 1; Exit and Herbiplus were not included in this set of experiments) at a constant pressure of 185 kPa from an air compressor (Balma® 50 L; 1.5 kW; www.balma.com), custom-fitted with activated carbon filters to remove any possible oil contamination. The spray gun was cleaned with 70% ethanol solution, flushed with distilled water and air-dried after each treatment. The wire mesh trays were cleaned with 70% ethanol solution between treatments. Leaves were sprayed separately per volume × treatment combination as experimental units. Post-run-off treated leaves were carefully hung to air dry after each spray whilst pre-run-off applications were carefully placed with sprayed side facing upward, on water soaked paper towels inside plastic containers (300×60×250 mm) (van Zyl *et al*., 2013). The experiment was repeated five times with each repeat serving as a replicate.

Deposition analysis

In a dark room, a single leaf was positioned in the middle of a red back-illuminated Perspex box (300×210×110 mm) to reduce any shadowing and to enhance edging of leaves in captured images during analysis. The leaf was covered with a glass pane (200×200×2 mm) and illuminated using an ultra-violet light source (UV-A; ≈ 365 nm; Labino Mid-Light; www.labino.com). Digital photos were taken in Canon RAW file format (.CR2 ≈ 10 MB) of the upper leaf surfaces using a Canon EOS 40D camera (www.canon.com) equipped with a 60 mm macro lens. The camera was attached to a tripod in a fixed position directly above the leaf. RAW image files were converted to 8-bit Exif-TIFF files (.TIF ≈ 30 MB; 3888×2592 pixels) with Digital Photo Professional version 3.1.0.0 (CANON INC.; www.canon.com) for digital image analysis (Image Pro Plus software version 7.0; Media Cybernetics, www.mediacy.com) to determine the deposition quantity and quality of the fluorescent particles per leaf (van Zyl *et al.*, 2013).

Deposition quantity

Similar to the methodology used in van Zyl *et al*. (2013), deposition quantity was measured as percent total leaf area covered by pigment particles (percentage fluorescent particle coverage; FPC%).

Deposition quality

For deposition quality assessment (van Zyl *et al.*, 2014), the leaf area was divided into equally-sized squares [100 × 100 pixels (10,000 pixels)]. Depending on the leaf size, this resulted in at least 20 to more than 250 individual squares per leaf, of which the percent area covered by fluorescent pigment particles was determined for each square. The Interquartile Coefficient of Dispersion (ICD%), a form of the Coefficient of Quartile Variation (CQV) (Bonnet, 2006) per

leaf [((3rd quartile – 1st quartile)/(3rd quartile + 1st quartile))*100] was used as a measure of deposition quality (variation in deposition distribution) per leaf, *i.e.* uniformity of deposition on an individual leaf surface. Low ICD% values were indicative of better deposition quality. As another measurement of deposition quality, protected leaf area (%) was determined by calculating the percent of 100 × 100 pixel squares per leaf that had a median value above that of the FPC_{50} (2.07 FPC%) benchmark (van Zyl *et al.*, 2013).

Copper residue analysis

Repetitions were grouped into two batches to allow sufficient biomass for copper residue analysis, which was done on each batch separately by an accredited analytical laboratory (SGS Analytical Laboratory, Somerset-West, South Africa). Briefly, analysis involved dry ashing of 1 g plant material in a crucible, which is digested (ashed) by heating in a muffle furnace (500°C for 4 h). The ash residue was then dissolved in an acid solution, filtered, diluted to a specific volume and copper ionic particle residue determined as mg kg^{-1} by inductively coupled plasma (ICP) spectrometer (Perkin-Elmer AAnalyst 400; www.perkinelmer.com). The mean inherent amount of Cu of the control leaves was subtracted from each treatment residue.

Deposition parameters and control of Alternaria brown spot following post-run-off adjuvant sprays

Spray application

To simulate deposition parameters as influenced by adjuvants under high-volume field application sprays, a post-run-off (3 mL) spray volume of copper oxychloride, yellow fluorescent pigment and deionised water were sprayed alone or in mixture with spray adjuvants at the recommended concentrations (Table 1). Methodology was the same as described previously. Leaves were carefully hung to air dry after each spray to simulate run-off under field conditions.

Deposition analysis

Deposition analysis was done to determine and evaluate deposition quantity and quality parameters as described previously. ABS control (%) as predicted by the FPC benchmark model [Control = 100*(1 – exp(-0.3346*FPC%))] (van Zyl *et al*., 2013) was calculated from deposition quantity data from each treatment.

Inoculum

An isolate of *A. alternata* was recovered from symptomatic mandarin leaves in Nelspruit (Mpumalanga, South Africa). It was single-spored and identified using conidium morphology (Simmons 1999a; 1999b). Pathogenicity tests on susceptible ‘Nova’ mandarin leaves confirmed it to be the tangerine pathotype of *A. alternata* (Whiteside, 1976). It was stored in

the Stellenbosch University culture collection (STE-U no. 6593). Cultures on potato dextrose agar (PDA; MERCK Biolab, Gauteng, South Africa) were incubated at 27°C for 7 to 14 days under 12-h light-dark cycle until abundant conidia were observed. Conidial suspensions were produced by pouring sterile water onto the PDA cultures and rubbing the surface gently with an L-shaped glass rod. The conidial suspension was filtered through 2 layers of cheesecloth and adjusted to 1×10^5 spores mL L^{-1} with the use of a haemocytometer. To prevent the loss of virulence of the isolate, it was regularly inoculated and re-isolated from non-treated 'Nova' mandarin leaves.

Inoculation with Alternaria alternata

Following deposition analysis, sprayed leaves were placed back into the containers and transported back to the spray chamber. Upper leaf surfaces of sprayed leaves were spray inoculated with pre-run-off volumes (0.3 mL) of 1×10^5 spores mL L^{-1} suspension of *A. alternata*. Spray inoculation was done in the same manner described for spray application. The spray inoculated leaves were placed on moistened paper towels and incubated in the plastic containers at high relative humidity (>95%) at 27°C in the dark for 48 h until pin-point necrotic lesions (< 2 mm in diameter) developed on the control treatment leaves.

Disease assessment

After an incubation period of *c.* 48 h that resulted in pin-point ABS lesions on the unsprayed control treatments (before lesions expanded and connected), the leaves were removed from the plastic containers and the midrib of the leaves excised by means of a scalpel, splitting the leaves in two halves. Each piece was then digitally photographed under white light on a white Perspex covered light box in exactly the same order as the leaves were previously photographed for deposition analysis. Digital photographs of the symptomatic leaves were taken in JPEG (.JPG) format. Each photograph was manually analysed with Image Pro Plus software version 7.0 to determine the percent symptomatic area per leaf. This was subsequently expressed as the percent disease control per leaf relative to unsprayed control leaves. After the leaves were photographed, they were stored in plastic bags at -20°C for copper residue analysis of each treatment. The experiment was repeated 18 times.

Copper residue analysis

Copper residue analysis was done in exact similar manner as described in previously, except that the 18 repeats were divided into three separate batches to allow sufficient biomass for analysis.

Statistical analysis

Deposition quantity (FPC%) and quality (ICD% per leaf; percent protected leaf area), copper residue, and percent control (actual and predicted) data were subjected to analysis of variance (ANOVA) seperately for the pre-run-off and post-run-off volume sprays. Tukey's honest significance difference (HSD) test was calculated to identify significant differences between treatments at a 95% confidence interval. For the post-run-off volume spray, Pearson's correlation was used to investigate any linear relation between copper residue, deposition quantity, quality and control parameters, while data were visualised in scatter plots to investigate any non-linear relation, in which case suitable non-linear regression was performed. All statistical analysis was done using statistical analysis software [Addinsoft XLSTAT Version 2013.1.01 (www.xlstat.com)], except for deposition quality, which was determined using SAS (www.SAS.com).

RESULTS

Evaluation of pre- and post-run-off application

Spray deposition

Distinct deposition patterns could be observed on leaves following the addition of the yellow fluorescent pigment and adjuvants to the spray mixture when illuminated under black (UV) light. Pre-run-off applications indicated very small to larger droplet remnants that were uniformly distributed on the leaf surface (Fig. 1), whereas post-run-off application showed non-uniform and streaking deposition patterns (Fig. 2). Break-Thru S240, Nu-Film-17 and Citrole100 realised similar pre-run-off deposition patterns to those of copper oxychloride and pigment alone (Fig. 1A, B, C, D), but with slightly larger droplet remnants than the control treatment. In these cases, intact droplet remnants of different sizes were uniformly distributed over the leaf surface, while the other adjuvant treatments realised a more wavelike film (Fig. 1F), or a combination thereof with distinct droplets (Fig. 1E, G). Entreé and Wetcit at pre-run-off volumes showed excessive film-forming, with wave- or blotch-like remnants of pigment at lower edges of films (Fig. 1F, G).

Post run-off spray mixtures (Fig. 2) retained distinct remnants of droplets that occasionally connected to form larger droplet stains. Droplets formed streaking run-off pathways from the stem in the direction of the leaf tip, before settling on the leaf surface, drying and forming annuli or 'ring' deposits. Copper oxychloride alone, Break-Thru S240 and Nu-Film-17 retained similar deposition patterns on sprayed leaves (Fig. 2A, B, C). Droplets formed separate elongated tearstain patterns running into one another, forming large deposits, downwards to the tip of the leaf. Smaller coffee stain deposits were observed between larger droplets, uniformly deposited between droplet streaking channels. Citrole100 and Herbiplus had similar deposition patterns to those of Break-Thru S240 and Nu-Film-17, but with larger and less streaky droplets (Fig. 2D, G). Instead of droplet formation, sprays with Villa51, Entreé, Wetcit and Exit formed an

almost even wavelike film with visibly less pigment deposition over the leaf surface, only occasionally forming pigment blotches, with streaking patterns down to the tip of the leaf (Fig. 2E, F, H, I). At the tips of these leaves pigment deposits following run-off were visible.

Deposition analysis

Deposition quantity

Analysis of variance of deposition quantity (FPC%) indicated significant main effects for spray volume ($P < 0.0001$) and treatment ($P = 0.012$). The pre-run-off spray volume had significantly higher deposition quantity than the post-run-off spray volume (4.88 *vs.* 2.36 FPC%). Nu-Film-17 (5.41 FPC%) retained significantly higher deposition quantity than Villa51 (3.09 FPC%), Wetcit (2.58 FPC%), Citrole100 (2.56 FPC%) and Entreé (2.04 FPC%) (results not shown). Even though not significant, the interaction for treatment × spray volume ($P = 0.975$) was discussed in order to describe these and other adjuvant effects at pre- and post-run-off volumes.

At pre-run-off sprays, the highest deposition quantities were realised by Nu-Film-17 (6.70 FPC%), Break-Thru S240 (6.48 FPC%) and copper oxychloride alone (6.42 FPC%). Wetcit (3.61 FPC%), Citrole100 (3.34 FPC%) and Entreé (3.12 FPC%) retained lower quantities, while Villa51 (4.52 FPC%) retained intermediate quantities. None of these treatments differed significantly from each other.

At post-run-off sprays, all sprays retained similar deposition quantity (4.13 to 1.60 FPC%), except for Entrée (0.96 FPC%), which realised the lowest deposition quantity. Nu-Film-17 and copper oxychloride alone had the highest deposition quantity (4.13 and 3.60 FPC%, respectively). Break-Thru S240 (2.83 FPC%) retained intermediate quantities of pigment, while lower quantities were retained by Villa51, Wetcit, Citrole100 (1.80 to 1.60 FPC%) (Table 2).

Deposition quality

Analysis of variance of deposition quality values (ICD%) indicated significant main effects for spray volume ($P < 0.0001$) and treatment ($P < 0.0001$). Significantly better deposition quality was realised with the pre-run-off spray compared with the post-run-off spray (38.27 *vs*. 60.86 ICD%). Nu-Film-17 (31.31 ICD%), copper oxychloride alone (37.88 ICD%) and Break-Thru S240 (44.09 ICD%) had significantly better deposition quality than the rest of the treatments (54.24 to 61.78 ICD%).

Although not significant, the interaction for treatment × spray volume ($P = 0.132$) was discussed to better describe the adjuvant effects at pre- and post-run-off volumes. Visual assessment of pigment deposition and distribution on leaves related well with deposition quality determined through image analysis (Fig. 1 and 2).

At pre-run-off, copper oxychloride alone treatment (21.82 ICD%) had the lowest variation in pigment distribution on leaf surfaces (best deposition quality), similar to that retained by

Break-Thru S240 (27.73 ICD%), Nu-Film-17 (29.12 ICD%), Citrole100 (37.74 ICD%), Villa51 (43.56 ICD%) and Wetcit (49.65 ICD%) sprays. Entreé (58.27 ICD%) realised markedly poorer deposition quality (Table 2).

At post-run-off application, the best deposition quality was realised by Nu-Film-17 (33.50 ICD%). Deposition quality was markedly poorer with copper oxychloride alone (53.94 ICD%), and for the other adjuvant treatments (60.46 to 74.34 ICD%) (Table 2).

Analysis of variance of protected leaf area (%) indicated significant main effects for volume (P = 0.0001) and treatment (P < 0.0001). On average, the pre-run-off spray had a significantly larger protected leaf area than the post-run-off spray (57.95 *vs.* 36.88%). Nu-Film-17, Break-Thru S240, Villa51 and Citrole100 (68.56 to 42.23%) realised similar protected leaf area than the copper oxychloride alone treatment (67.99%). Wetcit and Entreé (37.27 and 15.48% respectively) realised significantly lower protected leaf area than the copper oxychloride aloe treatment (results not shown). Similar to the other parameters, the interaction treatment × spray volume (P = 0.178) was discussed. All treatments had statistically similar protected leaf area (74.86 to 52.95%) at the pre-run-off volume, except for Entreé, which realised significantly lower protected leaf area (25.60%) than the copper oxychloride alone (74.86%) spray. At the post-run-off volume, copper oxychloride alone (61.11%), Nu-Film-17 (72.18%), Break-Thru S240 (48.39%) Citrole100 (31.51%) and Villa51 (22.56%) had similar protected leaf areas than what were achieved at the pre-run-off volume with the same treatments. The rest of the treatments had poorer protected leaf area (17.07 to 5.35%) (Table 2).

Copper residue analysis

Analysis of variance of Cu-residue data (mg kg^{-1}) indicated a significant interaction for treatment × spray volume (P < 0.0001). All treatments had higher Cu residues per treatment at the pre-run-off volume compared with post-run-off volume, except for Nu-Film-17 (189 *vs.* 237 mg kg^{-1}), which realised the opposite. At the pre-run-off spray volume, copper oxychloride alone had significantly higher Cu residue (387 mg kg^{-1}) than the adjuvant treatments (309 to 125 mg kg^{-1}). All adjuvant treatments differed significantly with Break-Thru S240 (309 mg kg^{-1}) and Citrole100 (263 mg kg^{-1}) the highest, and Wetcit (125 mg kg^{-1}) and Villa51 (125 mg kg^{-1}) the lowest. At the post-run-off spray volume, the highest Cu residue was realised by Nu-Film-17, Break-Thru S240 and Citrole100 (237, 236 and 236 mg kg^{-1}, respectively), significantly higher than all other treatments (210 to 99 mg kg^{-1}). Villa51 had the lowest Cu residue (99 mg kg^{-1}). Unfortunately, due to limited biomass, the residue levels were determined on pooled samples and correlations could not be analysed for individual treatments. Cu residue and deposition quantity had a moderate positive correlation at the post run-off volume (r = 0.504) and a weak correlation (r = 0.278) at pre-run-off volumes.

Deposition parameters and control of Alternaria brown spot following post-run-off adjuvant sprays

Deposition quantity

Analysis of variance of deposition quantity values (FPC%) indicated significant effects for treatments ($P < 0.0001$). Copper oxychloride and pigment alone (5.02 FPC%), and sprays with Break-Thru S240 (4.77 FPC%), Nu-Film-17 (4.60 FPC%), Citrole100 (4.18 FPC%) and Villa51 (3.06 FPC%) resulted in the highest deposition quantities. Entreé (2.40 FPC%), Herbiplus (2.20 FPC%) and Wetcit (2.17 FPC%) deposited lower deposition quantities, higher than Exit (1.28 FPC%) (Table 3).

Deposition quality

Analysis of variance of deposition quality values (ICD%) and protected leaf area (%) indicated significant effects for adjuvant treatments ($P < 0.0001$). The lowest ICD% values (best deposition quality) was realised by Exit (45.44%), copper oxychloride alone (48.12%), Nu-Film-17 (48.17%) and Break-Thru S240 (49.81%), with treatments not differing significantly. Villa51 (58.52%), Wetcit (58.58%) Citrole100 (62.36%), Herbiplus (64.66%) and Entreé (68.47%) realised significantly higher ICD% values (Table 3).

Break-Thru S240, Nu-Film-17 and copper oxychloride alone (62.97 to 61.51%) realised the largest protected leaf area (%). Citrole100, Villa51 and Herbiplus (51.99 to 35.40%) realised lower protected leaf area whilst Wetcit, Entreé and Exit realised the lowest protected leaf area (26.98 to 11.20%) (Table 3). For the combined treatments, a strong positive linear relationship was observed between protected leaf area (%) and deposition quantity (FPC%) ($r = 0.788$). Individually, sprays with Entreé and Wetcit indicated a strong positive linear relationship between protected leaf area and deposition quantity ($r = 0.877$ and $r = 0.776$, respectively).

Leaf infection analysis

Very small brown to black lesions (0.5 to 2 mm) with white to yellow halos were observed on the spray-inoculated leaf surfaces after ≈48 hours' incubation. The percent control achieved differed significantly between treatments ($P < 0.0001$). All adjuvant treatments (64.19 to 73.14%) achieved similar to better control than copper oxychloride alone (54.82%) (Table 3).

ABS control values indicated a moderate correlation with deposition quantity ($r = 0.527$) for combined treatments and very weak to moderate ($r = -0.070$ to 0.469) for individual treatments; except for Entreé ($r = 0.654$). Strong correlations were observed between control and deposition quality (ICD%) values in a combined dataset ($r = 0.643$), but not for treatments individually ($r = -0.050$ to 0.382), except for Entreé ($r = -0.806$). Correlations between control and protected leaf area (%) values were poor ($r < 0.468$), except for Entreé ($r = -0.839$). Non-

linear relationships were not observed for post-run-off spray applications between control, deposition quantity, quality and protected leaf area.

Control (%) as predicted by the FPC benchmark model (van Zyl *et al*., 2013) differed significantly between the adjuvant treatments ($P < 0.0001$). Predicted control for the combined treatments correlated strongly with actual control achieved ($r = 0.647$); however, only Entreé had a strong linear relationship ($r = 0.711$), while the rest of the treatments indicating very weak to moderate relationships ($r = 0.076$ to 0.487). It was clear that the FPC benchmark model under- and over-predicted control in most cases (Table 3). Control was over-predicted for the copper oxychloride alone treatment (78.82%), compared with the actual control achieved (54.82%). This was also the case for Break-Thru S240 (77.32% *vs*. 64.19%) and Nu-Film (74.73% *vs*. 64.65%) and Citrole100 (74.67% *vs.* 58.93%). Predicted control was most accurate for Villa51 (61.26% *vs*. 60.18%), while under-predicted for Entreé (54.29% *vs*. 73.13%), Herbiplus (51.52% *vs*. 61.53%), Wetcit (49.16% *vs*. 60.18%) and Exit (32.84% *vs*. 52.66%) (Table 3).

Copper residue analysis

Analysis of variance of Cu-residue data (mg kg^{-1}) indicated significant treatment effects ($P < 0.0001$). The highest Cu-residue level was obtained following sprays with Nu-Film-17 (201.73 mg kg^{-1}), significantly better than copper oxychloride alone (106.42 mg kg^{-1}) (Table 3). The rest of the adjuvant treatments realised statistically similar Cu residues as that of the copper oxychloride alone treatment (57.02 to 179.75 mg kg^{-1}). Water only had a mean Cu-residue of 8.87 mg kg^{-1}, which is indicative of the inherent Cu content of the leaves. Overall, Cu residue values correlated strongly with deposition quantity (FPC%) ($r = 0.693$) and with protected leaf area (%) values ($r = 0.736$) but correlated moderately with deposition quality (ICD%) and control achieved ($r = 0.396$ and $r = 0.459$, respectively).

DISCUSSION

Apart from the two previous chapters, little to no research exists on the influence of adjuvants on contact fungicide deposition in fruit trees, especially citrus leaves and fruit, which needs to be protected from fungal or bacterial infection. To our knowledge, this is the first study to evaluate adjuvant influence on deposition quantity and quality parameters on citrus leaves at pre- and post-run-off spray volumes. Furthermore, this study is a first to evaluate the effect of adjuvants with copper oxychloride, on disease control, specifically Alternaria brown spot. As was found in previous chapters, the deposition assessment protocol developed by van Zyl *et al*. (2013, 2014) proved to be useful in studying the effects that pre- and post-run-off sprays with copper oxychloride and adjuvants have on deposition parameters.

The size, speed and projection angle to the target of the droplet and surface characteristics of the target has a major effect on droplet impact (Boukhalfa *et al*., 2014; Zwertvaegher *et al*.,

2014; Mayo *et al*., 2015; Massinon *et al*., 2017). Adjuvants influence droplet formation and impaction followed by deflection, bounce, shattering and adhesion/retention on target surfaces (Holloway *et al*., 2000; Xu *et al*., 2011; Dorr *et al*., 2015; Mayo *et al*., 2015). Dorr *et al.* (2015) and Massinon *et al.* (2017) found that the primary outcome following impaction on easy to moderately wettable surfaces with smooth surfaces (e.g. avocado leaves) was droplet adhesion, whilst reflection, bounce and shattering was low due to the less complex leaf surface. The effect was also lower with the addition of a trisiloxane ethoxylate (similar to Break-Thru S240) and a vegetable oil based adjuvant (similar to Exit and Entreé). The more complex a target surface (leaves with hairs, trichomes, rough texture, protuberant veins and thick and complex wax layers), the harder it is to overcome the physical and chemical interactions between the droplet and the leaf surface (Smith *et al*., 2000; Dorr *et al*., 2015). Young mandarin leaves ('flush') used in this study can be regarded as easy to moderately wettable with a relatively smooth cuticular surface and newly developed wax layer (Baker *et al*., 1975). Older mandarin leaves with a more developed cuticle and wax layer might be more difficult to wet since it would be more difficult to overcome surface tension. Therefore, it should be noted that deposition characteristics on more mature mandarin leaves could differ to flush leaves observed in this study. This will also be the case for citrus fruit and target surfaces of other plant species (Zwervaegher *et al*., 2014).

The addition of adjuvants at the pre- (1 mL) and post- (3 mL) run-off volumes had a definite effect on visual deposition quantity and quality of the fluorescent pigment, as well as Cu residue loading. These effects can be ascribed to spray volume and differences in the type and application concentration of adjuvants, which in turn would have influenced droplet formation, size, impaction and ultimately deposition on the leaf surface (de Ruiter *et al*., 1990; Holloway *et al*., 2000; Xu *et al*., 2010; Dorr *et al*., 2015; Mayo *et al*., 2015). Through visual observation, three types of deposition patterns were observed at pre-run-off volumes: (1) distinct droplet remnants and annuli, slightly differing in size and uniformly distributed over the leaf surface; (2) wavelike film covering the whole leaf surface with pigment deposition pockets at different positions on the leaf, mostly at the leaf edge; and (3) a combination of 1 and 2. Copper oxychloride, Citrole100, Nu-Film-17 and Break-Thru S240 grouped into the first pattern type. Size of the annulus deposits of the copper oxychloride-only sprays appeared to be slightly smaller than those of the two adjuvant treatments. This can be ascribed to the adjuvants, lowering surface tension of the droplet and thus increasing the contact angle resulting in larger deposited droplets. However, the surface tension and thus, droplet contact angle was not lowered to such an extent that droplets coalesced to create a uniform film; most probably due to the adjuvant type and concentration. Entreé and Exit grouped into the second deposition pattern type with the wavelike film creation being ascribed to a higher product concentration or a higher spreading activity resulting in a film-wetting deposition pattern rather than distinct droplet remnants. Surface tension was reduced to such an extent that nearby droplets

coalesced and created a film covering the whole leaf. Wetcit and Villa51 fell into the third pattern type. The mixture of film-wetting and distinct droplet remnants might be ascribed to a wider droplet size range where smaller droplets caused distinct droplets and larger droplets that coalesced caused the wave-like film-wetting patterns (Butler Ellis and Tuck, 1999; Holloway *et al*., 2000; de Ruiter *et al*., 1990). These findings concurred with those described by Holloway *et al*. (2000) on other crop types.

Visual assessment of post run-off characteristics showed run-off patterns for all treatments. These were more prominent with the addition of adjuvants. Droplets following Break-Thru S240, Citrole100 and Nu-Film sprays coalesced, forming running streaks to the tip of the leaf blade, but still retained uniformly distributed “coffee stain” pigment deposits over the leaf surface. Run-off patterns of Villa51, Entreé, Wetcit and Exit sprays were clearly aggravated by the adjuvants’ activity, since pigment deposits were wavelike in pattern, and in cases like Exit only faintly visible on the surface.

Adjuvants generally did not improve deposition quantity and quality significantly compared with the copper oxychloride alone application; in fact, deposition was in some cases significantly poorer. Deposition parameters were similar for adjuvants with similar deposition pattern types. The findings of Fourie *et al*. (2009) on the detrimental effects of run-off on deposition were supported as pre-run-off sprays retained higher deposition quantities at more uniform quality than post-run-off sprays. Van Zyl *et al.* (2010a) evaluated similar adjuvants (Break-Thru S240, Nu-Film-17, Villa51 and Wetcit) on grapevine leaves and found that these adjuvant sprays did not improve deposition quantity or quality on upper and lower leaf surfaces at a pre-run-off spray volume. Van Zyl *et al*. (2010a) measured deposition quantity and quality parameters on microscopic images of the leaf surface as the amount of deposited pigment (quantity) and the mean distance between pigment particles (quality), whereas deposition quantity and quality were determined on whole leaf surface images in the current study. Holloway *et al*. (2000) also found that organosilicones (similar to Break-Thru S240), terpine oil/resins (similar to Nu-Film-17) and vegetable oil adjuvants (similar to Entreé and Exit) did not significantly improve retention performance (deposition quantity) in relation to the control treatment on difficult-to-wet leaf surfaces of barley, pea and wheat. In our study, certain adjuvants (Nu-Film-17 and Break-Thru S240) gave similar deposition patterns to that of the copper oxychloride control treatment. In these cases, adjuvant dosage might have been sub-optimal for the active/type used, hence the limited effects observed. However, for adjuvant treatments where surface tension was reduced to the extent of droplet coalescing or spreading to a thin film to cover the leaf surface (Xu *et al*., 2011), we mostly measured poorer deposition. This was contradictory to the reported improved deposition parameters by adjuvant treatments (Holloway *et al*., 2000; Gaskin *et al*., 2005). In the cases where significant reduction in deposition parameters were observed (Entreé, Herbiplus, Wetcit and Exit), it can most possibly be ascribed to adjuvant activity at super-optimal dosages (*i.e.* spray volume and adjuvant

concentration) leading to droplet coalescing and run-off. Results might differ at similar concentrations used on other types and ages of targets, since the wettability of these targets, due to surface structure and composition will differ (De Ruiter *et al*., 1990).

All treatments at the post-run-off volume realised poorer deposition parameters than at the pre-run-off volume; retention ranged from 31% to 61% of the deposition quantity following pre-run-off sprays, with Nu-Film-17 (61%), copper only control (56%) and Citrole100 (53%) performing markedly better than the other adjuvant treatments (<44%). Post run-off sprays for the bio-efficacy trial indicated fairly similar deposition results. The type of adjuvant reduced dynamic surface tension to a certain extent that exacerbated run-off and reduced retention (Holloway *et al*., 2000), particularly when considering the 30° spraying angle and vertical hanging of leaves in our trials. Mayo *et al.* (2015) found that large amounts of droplets on leaf surfaces (as in the case of post-run-off volumes) has the potential to coalesce and form larger droplets, which then, due to gravitational influence, will aggravate run-off and through it will reduce retention capability and increased variation in chemical deposition in the run-off droplets' wake. In certain cases, adjuvant concentration might have a more drastic effect on deposition (Fears and Pontzen, 2008), resulting in excessive spreading (de Ruiter *et al*., 1990; Holloway *et al*., 2000). Xu *et al.* (2015) indicated the positive effect increasing concentrations of non-ionic surfactants, modified seed oils and mineral oils can have on spread ability of droplets on difficult-to-wet, waxy and hairy leaves. This was not the case on easily to moderately wettable citrus leaves used in this study. For example, Exit, which is the same formulation as Entreé, but has a higher application concentration (2.5 mL L^{-1} *vs.* 0.6 mL L^{-1}, respectively), deposited pigment quantities almost half of that of Entreé, indicating a definite adjuvant concentration and volume effect on deposition quantity.

As was found with deposition quantity, quality and protected leaf area, Cu residues were higher at pre-run-off volumes than at post run-off volumes. Previous studies have also reported a decrease in copper oxychloride with increasing spray volume due to run-off in citrus orchard spray trials (Farooq and Salyani, 2002) and higher copper tracer deposits with lower spray volumes (Salyani and Whitney, 1988; Salyani and McCoy, 1989). It was postulated that larger copper particles with lower tenacity coefficients were more readily washed off, resulting in lower copper residue levels at higher spray volumes (Somers, 1956; Somers and Thomas, 1956). Copper formulations with smaller particle sizes retained higher residues and longer residual effects on citrus leaves (Vicent *et al.,* 2007; Schutte *et al.* 2012). Logically, higher copper residues as a result of better retention should improve disease control. Furthermore, better deposition quality achieved due to the addition of adjuvants as found in this study, will also improve disease control due to better distribution of particles over the leaf surface (van Zyl *et al.*, 2010a). It should be noted that residue loading, penetration or absorbsion will possibly differ when adjuvants are used with systemic fungicides compared to inert contact fungicides, such as copper oxychloride and mancozeb formulations (Rossouw *et al*., 2018).

Studies have shown to improve absorbsion of systemic funigicdes. Gent *et al*. (2003) found an 30% increase of azoxystrobin absorbsion on onions an a 21% absorbsion increase on potatoes with the addition of organosilicone/methelyated seed oil-based adjuvant to sprays and a 41% azoxystrobin absorbsion increase on onions and a 39% increase on dry bean with the addition of a wetter/sticker adjuvant combination to sprays.

In the present study, Cu residue values were weakly correlated with deposition quantity values at pre-run-off spray volumes. At post-run-off volumes, a moderate to strong positive correlation was observed between Cu residue and deposition quantity This apparent anomaly between pigment quantity and Cu residue results contradicts what was found by van Zyl *et al.* (2013), who found a very strong linear correlation between pigment quantity and Cu residue at a pre-run-off volume of 0.5 mL. Schutte *et al.* (2012) evaluated the retention and persistence of different copper formulations on orange fruit and leaves and also found good to strong correlations between copper residue measured and deposition quantity at various industry spray volumes in orchard trials. Rossouw *et al.* (2018) also found a very strong correlation between different formulations of mancozeb and deposition quantity using the same yellow fluorescent pigment on apple seedling leaves. These studies all found that the yellow fluorescent pigment was a good tracer for contact fungicides evaluated. However, none of the mentioned studies evaluated adjuvants. At pre-run-off volumes in the current study, adjuvants either improved Cu residue retained, as was found with Nu-Film-17, Break-Thru S240 and Citrole100 at the pre-run-off spray volume, or adjuvants worsened run-off and therefore loss of Cu as was found with Entrée, Villa51 and Wetcit. Post run-off sprays improved Cu residue and deposition quantity retained, as was found with Nu-Film-17, Break-Thru S240 and Citrole100 in relation to adjuvants Entrée, Villa51 and Wetcit, which reduced Cu-residue and deposition quantity retained due to run-off. Nonetheless, one would have expected that the fluorescent pigment would act as a good tracer regardless of spray volume used and any degree of run-off. Poor correlations and inconsistent outcomes between deposition quantity and Cu residues with the addition of adjuvants can possibly be ascribed to adjuvants disturbing the physical structure of the cuticle layer (Knoche *et al*., 1992; Zabkiewicz, 2007), directly influencing the amount of active ingredient deposited and retained before and after spray run-off. Adjuvants are known to physically influence surface microstructures such as cuticular foldings and epiculticular waxes that minimise contact area between the spray droplet and the target surface (Wagner *et al*., 2003; Bargel *et al*., 2006) to increase deposition and retention (Hall *et al*., 1998). Research on the physical and chemical effects of adjuvants on the leaf cuticle was not investigated in this study and how this influence deposition should be investigated in future studies.

The addition of adjuvants to post-run-off copper oxychloride sprays marginally improved or realised similar control of ABS compared with the copper oxychloride alone control treatment. A relatively poor correlation between ABS control and copper residue levels (or

pigment deposition quantity) was observed, which indicate that deposition quantity alone was not a reliable predictor of control achieved in these cases, despite being accurate in predicting ABS control at pre-run-off sprays with copper oxychloride alone (van Zyl *et al.*, 2013). For example, the copper oxychloride control spray retained a deposition quantity of 5.02 FPC% (106.42 mg kg^{-1} Cu), more than double that of Entrée, 2.40 FPC% (65.59 mg kg^{-1} Cu). However, copper oxychloride sprays with Entrée achieved 73.13% control compared with 54.82% control achieved with sprays of copper oxychloride alone. Predicted control values at these deposition quantity values seemed to be under predicted in the case of Entrée (54.64%), and over predicted with copper oxychloride alone (78.26%). Van Zyl *et al*. (2010a) attributed improved *B. cinerea* control on grapevine leaves to improved deposition quality of the fungicide at a microscopic level (10× magnification). However, we observed a poor correlation between deposition quality and ABS control in our study where deposition quality was assessed at a macroscopic scale; for example, Entreé had the poorest deposition quality, but the best ABS control. Improved imaging sensitivity might address the limitations of assessing deposition quality on a macroscopic scale. ABS control at pre-run-off spray volumes were not evaluated and should be focussed on in future studies. ABS control at pre-run-off volumes might be better due to less run-off resulting in better deposition quantity, quality and Cu residue loading (Fourie *et al.*, 2009; this study).

Hazen (2000) postulated that adjuvants with wetter-spreader-penetrant activity (such as Exit and Entrée) might increase infection due to disturbance or softening of the cuticle wax layer, making pathogen invasion easier. However, we observed the contrary, with Entreé realising markedly improved ABS control.

The FPC benchmark model (van Zyl *et al.*, 2013) was built with only one deposition parameter, *i.e.* deposition quantity, and did not account for the effect of deposition quality on disease control. The further improvement of this model with the addition of a reliable quality parameter was highlighted in this study. McMillan (1970) found the addition of Nu-Film-17 to copper sprays resulted in improved control of avocado scab. This was also the case with Nu-Film-17 evaluated together with mancozeb for the control of cucumber spot (Blazques and McGrew, 1969). These studies could not explain the reason for improved disease control. Gent *et al*. (2003) presumed that increased protectant activity with the protectant fungicide maneb of early blight of potato and rust of dry-bean was largely due to improved fungicide coverage, but could not fully explain the results obtained. The anomalous results from our study, *i.e.* poor deposition parameters but good disease control, can be ascribed to either the adjuvant effects on deposition parameters (specifically microscopic deposition quality) (Gent *et al*., 2003; Knoche *et al*., 1992), or possibly direct adjuvant effects on pathogen development (van Zyl *et al*., 2010b), or potential synergistic effects between adjuvant and fungicide (Orbovic, 2007). These aspects need to be investigated further.

Whilst ABS control was similar in adjuvant treatments, it should be considered that in our study leaves were inoculated with the pathogen on the same day as spray application. Under field conditions, copper oxychloride is registered to provide up to 5 weeks' protection against a disease such as ABS. Vicent *et al.* (2009) evaluated reduced concentration copper sprays on mandarin fruit and the effects on ABS disease control. They found that reduced concentration sprays still effectively controlled ABS in a programme with 28-day spray intervals, but noted that very little rain fell over the test period and that the reduced concentrations might not be effective in high rainfall and windy areas. Schutte *et al.* (2012) evaluated weathering curves of different copper formulations on orange fruit surfaces. They found that initial copper deposits reduced by 48% to 60% within the first 2 weeks after application, followed by a more gradual decline (24% to 41%) from 14 to 56 days. This reduction was ascribed to accumulative rainfall (wash-off), weathering and to a lesser extent fruit expansion over time. Due to the reduction in copper deposit over time, the initial deposition is important for adequate protection as time passes after application. In our study, certain adjuvant treatments caused a significant reduction in Cu residue and the effect thereof on long-term protection should be investigated. Thus, in a real-world scenario, initial copper deposits following these treatments might be too low to provide sufficient protection during a 35-day spray interval.

As was found in the two previous studies (Chapters 3 and 4), adjuvants have the potential benefit to improve deposition parameters. However, these studies, as confirmed in the present study, also demonstrated the negative effects adjuvants can have on deposition parameters and residue loading if used at post-run-off volumes and too high product concentrations. The potential improvements of deposition parameters and residue loading therefore depend on the interaction between spray volume and product concentration and possibly target surface characteristics. Despite the effect on deposition quantity and Cu residue retention, adjuvant treatments generally gave similar or improved, however not significantly, ABS control on leaf surfaces. Even so, the negative effect of certain adjuvants on Cu residue loading on target surfaces should be of concern since it is unknown if these reduced initial deposits would be sufficient to realise sustained protection over time, or until the next protectant spray is applied, especially taking into account rain wash-off, weathering, fruit/leaf expansion under field conditions.

ACKNOWLEDGEMENTS

The authors acknowledge Citrus Research International and THRIP for funding and Marieta Van Der Rijst (ARC-Biomet, Stellenbosch) for procedural and statistical support in deposition quality measurements. The first author also acknowledges the National Research Foundation, Bayer CropScience South Africa and the Department of Plant Pathology, Stellenbosch University for bursary funding.

REFERENCES

Akimitsu, K., Peever, T.L., Timmer, L.W., 2003. Molecular, ecological and evolutionary approaches to understanding Alternaria diseases of citrus. Molecular Plant Pathology 4: 435-446.

Albrigo, L.G., Beck, H.W., Timmer, L.W., Townsend, K., 1997. Copper fungicides-Residues for disease control and potential spray burn. Proceedings of the Florida State Horticultural Society 110: 67-70.

Alva, A.K., Graham, J.H., 1991. The role of copper in citriculture. Advances in Agronomy 1: 145-170.

Baker, A.B., Procopiou, J., Hunt, M.H., 1975. The cuticles of Citrus species. Composition of leaf and fruit waxes. Journal of the Science of Food and Agriculture 26: 1093-1101.

Bargel, H., Cerman, Z., Koch., Koch, K., Netnhuts, C., 2006. Structure-function relationships of the plant cuticle and cuticular waxes – a smart material? Functional Plant Biology 33: 893-910.

Behlau, F., Belasque Jr., Bergamin Filho, A., J., Graham, J.H., Leite Jr., R.P., Gottwald, T.R., 2008. Copper sprays and windbreaks for control of citrus canker on young orange trees in southern Brazil. Crop Protection 27: 807-813.

Behlau, F., Belasque Jr., J., Graham, J.H., Leite Jr., R.P., 2010., Effect of frequency of copper applications on control of citrus canker and the yield of young bearing sweet orange trees. Crop Protection 29: 300-305.

Blazques, C.H., McGrew, G.T., 1969. Effect of Nu-Film 17 on fungicides evaluated for control of cucumber target spot. Proceedings of the Florida State Horticultural Society 3378: 141-143.

Bonnet, D.G., 2006. Confidence interval for a coefficient of quartile variation. Computational Statistics and Data Analysis 50: 2953-2957.

Boukhalfa, H.H., Massinon, M., Belhamra, M., Lebeau, F. 2014. Contribution of spray droplet pinning fragmentation to canopy retention. Crop Protection 56: 91-97.

Brink, J.C., Holz, G., Calitz, F.J., Fourie, P.H., 2004. Development of a protocol to quantify spray deposits on grape bunches. 7th International Symposium on Adjuvants for Agrochemicals (*ISAA 2004*), South Africa, 230-236.

Brink, J.C., Holz, G., Fourie, P.H., 2006. Effect of fungicide spray cover on *Botrytis cinerea* infection in grape bunches. South African Journal for Enology and Viticulture 27: 51-56.

Burghardt, M., Riederer, M., 2006. Cuticular transpiration. In: Riederer, M., Műller, C. (Eds.), Biology of the plant cuticle. Blackwell Pub. Annual Review of Plant Biology. 23: 292-309.

Butler Ellis, M.C. and Tuck, C.R. 1999. How adjuvant influence spray formation with different hydraulic nozzles. Crop Protection 18: 101-109.

Butler Ellis, M.C., Tuck, C.R. and Miller P.C.H. 1997. The effect of some adjuvants on sprays produced by agricultural flat fan nozzles. Crop Protection 18: 41-50.

Craver, T.L.W., Gurr, S.J., 2006. Filamentous fungi on plant surfaces. In: Riederer, M., Műller, C., (Eds.), Biology of the plant cuticle. Blackwell Pub. Annual Review of Plant Biology 23: 306-392.

Cunningham, G.P., Harden, J., 1999. Sprayers to reduce spray volumes in mature citrus trees. Crop Protection 18: 275-281.

Cunningham, G.P., Harden, J., 1998a. Air-tower sprayers increase spray application efficiency in mature citrus trees. Australian Journal of Experimental Agriculture 38: 871-877.

Cunningham, G.P., Harden, J., 1998b. Reducing spray volumes applied to mature citrus trees. Crop Protection 17: 289-292.

de Ruiter, H., Uffing, A.J.M., Meinen, E., Prins, A., 1990. Influence of surfactants and plant species on leaf retention of spray solutions. Weed Science 38: 567-572.

Dewdney, M.M., Graham, J.H., 2012. 2012 Florida citrus pest management guide: Citrus canker. PP-182, series of the Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu.

Dewdney, M.M., Schubert, T.S., Estes, M.R., Peres, N.A., 2012 Florida citrus pest management guide: Citrus black spot. PP-279, series of the Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu.

Dewdney, M.M., Timmer, L.W., 2012. 2012 Florida citrus pest management guide: Greasy spot. PP-144, series of the Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu.

Dewdney, M.M., Timmer, L.W., 2012. 2012 Florida citrus pest management guide: Melanose. PP-145, series of the Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu.

Dewdney, M.M., Timmer, L.W., 2012. 2012 Florida citrus pest management guide: Citrus Scab. PP-146, series of the Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu.

Dewdney, M.M., Timmer, L.W., 2012. 2012 Florida citrus pest management guide: Alternaria brown spot. PP-147, series of the Plant Pathology Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu.

Dorr, G.J., Wang, S., Mayo, L.C., McCue, S.W., Forster, W.A., Hanan, J., He, X. 2015. Impaction of spray droplets on leaves: influence of formulation and leaf character on shatter, bounce and adhesion. Experiments in Fluids 56:1-17.

Elena, K., 2006. Alternaria brown spot of Minneola in Greece; evaluation of citrus species susceptibility. European Journal Plant Pathology 115: 259-262.

Faers, M.A., Pontzen, R., 2008. Factors influencing the association between active ingredient and adjuvant in the leaf deposit of adjuvant-containing suspoemulsion formulations. Pest Management Science 64: 820-833.

Fourie, P.H., du Preez., M., Brink, J.C., Schutte, G.C., 2009. The effect of runoff on spray deposition and control of Alternaria brown spot of mandarins. Australasian Plant Pathology 38: 173-182.

Furness, G.O., Drew, H.J., Magarey, P.A., Miller, P.H., 1998. Fruit tree and vine sprayer calibration based on canopy size and length of row: Unit canopy row method. Crop Protection 17: 639-644.

Furness, G.O., Manktelow, D.W.L., Thompson, A.J., 2006a. A Visual droplet number rating chart and fluorescent pigment sprays to estimate chemical deposition and spray coverage on plant foliage. Aspects of Applied Biology 77: 171-178.

Furness, G.O., Manktelow, D.W.L., Thompson, A.J., 2006b. Multi-fan spray towers to improve dose efficiency and spray coverage uniformity in citrus trees. Aspects of Applied Biology 77: 481-488.

Gaskin, R. E., Elliott, G.S., Manktelow, D.W.L., Steele, K.D., 2006. Adjuvant and application technologies to minimise off-target drift from kiwifruit sprays. New Zealand Plant Protection 59: 217-222.

Gaskin, R.E., Elliott, G.S., Manktelow, D.W.L., Skinner, S.J., 2004. Use of a superspreader adjuvant to reduce spray application volumes on avocados. New Zealand Plant Protection 57: 266-270.

Gaskin, R.E., Forster, W.A., Steele, K.D., 2005. Characterising plant surfaces for spray adhesion and retention. New Zealand Plant Protection 58: 179-183.

Gaskin, R.E., Manktelow, D.W.L., Pak, H., 2008. Improving spray distribution and coverage on avocado trees in low volume, concentrate sprays. New Zealand Plant Protection 61: 147-152.

Gaskin, R.E., Steele, K.D., 2009. A comparison of sticker adjuvants for their effects on retention and rianfastning of fungicide sprays. New Zealand Plant Protection 62: 339-342.

Gent, D.H., Schwartz, H.F., Nissen, S.J., 2003. Effect of commercial adjuvants on vegetable crop fungicide coverage, absorbtion and efficacy. Plant Disease 87: 591-597.

Gottwald, T.R., Graham, J.H., Riley, T.D., 1997. The influence of spray adjuvants on exacerbation of citrus bacterial spot. Plant Disease 81: 1305-1310.

Green, J.M., Beestman, G.B., 2007. Recently patented and commercialized formulation and adjuvant technology. Crop Protection 26: 320-327.

Grout, T.G., 1997. Spray volumes and coverage requirements for citrus in South Africa. Citrus Journal 31, 19-20.

Grout, T.G., 2003. Use of plant protection products. Section 2.7 in: Grout, T.G. (Ed.), Citrus production guidelines. Integrated Pest Management. Vol III. Citrus Research International, Nelspruit, South Africa.

Hall, F.R., Cooper, J.A., Ferree, D.C., 1991. Orchard geometry and pesticide placement. BCPC Monograph 46: 171-176.

Hall, J.H., Holloway, P.J., Stock., D. 1998. Factors affecting foliar retention of some model adjuvant oil-in-water emulsions. Pesticide Science; Extended Summaries: 9th Pesticide Chemistry Congress: 320-322.

Hazen, J. L., 2000. Adjuvants-Terminology, classification, and chemistry. Weed Technology 14: 773-784.

Holloway, P.J. 1969. Chemistry of leaf waxes in relation to wetting. Journal of the Science of Food and Agriculture 20: 124-128.

Holloway, P.J., Butler Ellis, M.C., Webb, D.A., Western, N.M., Tuck, C.R., Hayes, A.L. and Miller, P.C.H. 2000. Effects of some agricultural tank-mix adjuvants on the deposition efficiency of aqueous sprays on foliage. Crop Protection 19: 27-37.

Holownicki, R., Doruchowski G., Świechowski, W. And Jaeken, P. 2002. Methods of evaluation of spray deposit and coverage on artificial targets. Electronic Journal of Polish Agricultural Universities (EJPAU)-Agricultural Engineering 5.

Hunsche, M., Damerow, L., Schmitz-Eiberger, M., Noga, G., 2006. Leaf surface characteristics of apple seedlings, bean seedlings and kohlrabi plants and their impact on the retention and rainfastness of mancozeb. Pest Management Science 62: 839-847.

Hunsche, M., Alexeenko, A., Damerow, L., Noga, G., 2011. Rain-induced removal of copper from apple leaves: Influence of rain properties and tank-mix adjuvants on deposit characteristics at the micro-scale. Crop Protection 30: 495-501.

Jejčič, V., Godeša, T., Hočevar, M., Širok, B., Malneršič, A., Štancar, A., Lešnik, M., Stajnko, D., 2011. Design and testing of an ultrasound system for targeted spraying in orchards. Strojniški vestnik – Journal of Mechanical Engineering 57: 587-598.

Kiely, T.B., 1964. Brown spot of Emperor mandarin. The agricultural gazette of New South Wales 75: 854-856.

Knoche, M., Lenz, F., Noga, G., 1992. Surfactant-induced phytotoxicity: evidence for interaction with epicuticular wax fine structure. Crop Protection 11: 51-56.

Koch, H., Knewitz, H., 2008. Methodology and sampling technique of spray deposit and distribution measurement in vineyards. Nachrichtenblatt des Deutschen Pflanzenschutzdienstes 60: 25-30.

Koch, K., Ensikat, H., 2008. The hydrophobic coatings of plant surfaces: Epicuticular wax crystals and their morphologies, crystallinity and molecular self-assembly. Micron 39, 759-772.

Kohmoto, K., Akimitsu, K., Otani, H., 1991. Correlation of resistance and susceptibility of citrus to *Alternaria alternata* with sensitivity to host-specific toxins. Phytopathology 81: 719-722.

Kohmoto, K., Itoh, Y., Shimomura, N., Kondoh, Y., Otani, H., Kodama, M., Nishimura, S., Nakatsuka, S., 1993. Isolation and biological activities of two host-specific toxins from the tangerine pathotype of *Alternaria alternata*. Phytopathology 83: 495-502.

Kohmoto, K., Scheffer, R.P., Whiteside, J.O., 1979. Host-selective toxins from *Alternaria citri*. Phytopathology 69: 667-671.

Kotzé, J.M., 1981. Epidemiology and control of citrus black spot in South Africa. Plant Dis. 65: 945-950.

Kotzé, J.M., 2000. Citrus black spot. Pages 23-25 in: Compendium of Citrus Diseases (second Edition). Timmer, L.W., Garnsey, S.M. and Graham, J.H. APS Press, The American Phytopathological Society, St. Paul Minnesota, USA.

Lin, C-H., Ling Yang, S., Chung, K., 2009. The YAP1 homolog-mediated oxidative stress tolerance is crucial for pathogenicity of the necrotrofic fungus *Alternaria alternata* in citrus. Molecular Plant-Microbe Interactions 22: 942-952.

Massinon, M., De Cock, N., Forster, A.W., Nairn, J.J., McCue, S.W., Zabkiewics, J.A., Lebeau, F. 2017. Spray droplet impaction outcomes for different plant species and spray formulations. Crop Protection 99: 65-75.

Mayo, L.C., McCue, S.W., Moroney, T.J., Forster, W.A., Kempthorne, D.M., Belward, J.A., Turner, I.W. 2015. Simulating droplet motion on virtual leaf surfaces. Royal Society of Open Science 2: 140528

McMillan Jr., R.T., 1970. Effectiveness of copper when combined with Nu-Film 17 for control of avocado scab. Proceedings of the Florida State Horticultural Society 83: 386-388.

Miles, A.K., Cooke, A.W., Willingham, S.L., 2005. Field evaluation of plant activator, captan chlorothalonil, copper hydroxide, iprodione, mancozeb, and strobilurins for the control of citrus brown spot of mandarin. Australasian Plant Pathology 34: 63-71.

Mondal, S.N., Reis, R.F., Timmer, L.W., Vicent, A., 2007. Efficacy of pre- and postinoculation application of fungicides to expanding young citrus leaves for control of melanose, scab, and alternaria brown spot. Plant Disease 91: 1600-1606.

Orbovic, V., Achor, D., Syversten, P., 2007. Adjuvants affect penetration of copper through isolated cuticles of citrus leaves and fruit. HortScience 42: 1405-1408.

Reis, R.F., de Goes, A., Mondal, S.N., Shilts, T., Brentu, F.C., Timmer, L.W., 2006. Effect of lesion age, humidity, and fungicide application on sporulation of *Alternaria alternata*, the cause of brown spot of tangerine. Plant Disease 90: 1051-1054.

Riederer, M., Schneider, G., 1990. The effect of the environment on the permeability and composition of *citrus* leaf cuticles. Planta 180: 154-165.

Rogiers, S.Y., Whitelaw-Weckert, M., Radovanonic-Tesic, M., Greer, L.A, White, R.G., Steel, C.C., 2005. Effects of spray adjuvants on grape (*Vitis vinifera*) berry microflora, epicuticular wax and susceptibility to infection by *Botrytis cinerea*. Australas. Plant Pathology 34: 221-228.

Rossouw, C.J., Fourie, P.H., van Zyl., J.G., Hoffman, J.E., McLeod, A. 2018. Rainfastness of mancozeb on apple seedlings determined through deposition quantification of mancozeb residue and fluorescent pigment. Crop Protection 106: 93-102.

Rudgard, S.A., Pettitt, T.R., Hadley, P., 1990. Tenacity, biological activity and redistribution of copper fungicides on cocoa in controlled environments. Crop Protection 9: 281-288.

Salyani, M., 2005. 2005 Florida citrus pest management guide: Pesticide Application Technology. AE-259, series of the Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu.

Salyani, M., 2006. 2006 Florida citrus pest management guide: Pesticide Application Technology. ABE-356, series of the Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu

Salyani, M., Farooq, M., 2004. Drift potential of citrus air-carrier sprayers. Proceedings of the Florida State Horticultural Society 117: 130-135.

Salyani, M., Hoffmann, W.C., 1996. Effects of application time and spray volume on deposition. Proceedings of the Florida State Horticultural Society 109: 46-50.

Salyani, M., McCoy, C.W., 1989. Deposition of different spray volumes on citrus trees. Proceedings of the Florida State Horticultural Society 102: 32-36.

Salyani, M., Whitney, J.D., 1988. Evaluation of methodologies for field studies of spray deposition. Transactions of American Society of Agricultural and Biological Engineers 31: 390-395.

Salyani, M., Whitney, J.D., 1990. Ground speed effect on spray deposition inside citrus trees. Transactions of the American Society of Agricultural and Biological Engineers 33: 361-366.

Salyani, M., Farooq, M., Sweeb, R.D., 2007. Spray deposition and mass balance in citrus orchard applications. Transactions of the American Society of Agricultural and Biological Engineers 50: 1963-1969.

Schutte, G.C., Beeton, K.V., Kotzé, J.M., 1997. Rind stippling on Valencia oranges by copper fungicides used for control of citrus black spot in South Africa. Plant Disease 81: 851-854.

Schutte, G.C., Lesar, K.H., Pelser, P. du T., 1992. The use of tebuconazole for the control of *Alternaria alternata* on 'Minneola Tangelos and its potential to control postharvest decay when applied as a preharvest spray. Proceedings of the International Society of Citriculture 3: 1070-1074.

Schutte, G.C., Kotze, C., van Zyl, J.G., Fourie, P.H., 2012. Assessment of retention and persistence of copper fungicides on orange fruit and leaves using fluorometry and copper residue analyses. Crop Protection 42: 1-9.

Shanmugam, V., Dhyani, D. and Ananthapadmanaban, D. 2011. First report of *Alternaria* sp. causing blight on *Incarvillea emodi*. Australasian Plant Disease Notes 6: 33-35.

Simmons, E.G. 1999a. Alternaria themes and variations – Classification of citrus pathogens. Mycotaxon 70: 226-235.

Simmons, E.G. 1999b. Alternaria themes and variations – Host-specific toxin producers. Mycotaxon 70: 236-243.

Solel, Z., 1991. Alternaria brown spot on Minneola tangelos in Israel. Plant Pathology 40: 145-147.

Solel, Z., Kimchi, Miriam., Oren, Y., 1997. Control of Alternaria brown spot of Minneola tangelo with fungicides. Crop Protection 7: 695-664.

Somers, T., 1956. Studies of spray deposits. I. – Effect of spray supplements on the tenacity of copper fungicides. Journal of the Science of Food and Agriculture 7: 160-172.

Somers, T., Thomas, W. D. E., 1956. Studies of spray deposits. II. – The tenacity of Fungicides on artificial and leaf surfaces. Journal of the Science of Food and Agriculture 10: 655-667.

Smith, D.B., Askew, S.D., Morris, W.H., Shaw, D.R., Boyette, M. 2000. Droplet size and leaf morphology effects on pesticide spray deposition. Transactions of the American Society of Agricultural Engineers 43: 255-259.

Spanoghe, P., De Schampheleire, M., Van der Meeren, P., Steurbaut, W., 2007. Influence of agricultural adjuvants on droplet spectra. Pest Management Science 63: 4-16.

Stock, D., Briggs, G., 2000. Physicochemical properties of adjuvants: Values and Applications. Weed Technology 14: 798-806.

Stover, E., McClure, P., Salvatore, J., 2002. Influence of spray volume, ground speed, and a sensor-actuated spray controller on melanose control and fruit marking in fresh Indian river grapefruit. Proceedings of the Florida State Horticultural Society 115: 1-5.

Stover, E., Scotto, D., Wilson, C., Salyani, M., 2002a. Spray application to citrus: Overview of factors influencing spraying efficacy and off-target deposition. HS-851, series of the Horticultural Sciences Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. http://edis.ifas.ufl.edu.

Sundaram, K.M.S., Sundaram, A., 1987. Role of formulation ingredients and physical properties on droplet size spectra, deposition, and persistence of aerially sprayed

amonicarb and mexacarbate in forest litter soil samples. Pages 139-151 in: Pesticide Formulations and Application Systems: Seventh Volume (G. B. Beestman and D.I.B Vander Hooven, Eds). American Society for Testing and Materials, Philadelphia, United States of America.

Swart, S.H., Schutte, G.C., Swart, W.J., Wingfield, M.J., 1998. Chemical control of Alternaria brown spot on Minneola tangelo in South Africa. Annals of applied Biology 133: 17-30.

Timmer, L.W., Albrigo, L.G., Zitko, S.E., 1998. Split applications of copper fungicides improve control of melanose on Grapefruit in Florida. Plant Disease 82: 983-986.

Timmer, L.W., Peever, T.L., Solel, Z. And Akimitsu, K. 2003. Alternaria diseases of citrus - Novel pathosystems. Phytopathologia Mediterranea 42: 3-16.

Timmer, L.W., Zitko, S.E., 1997. Evaluation of copper fungicides and rates of metallic copper for control of melanose on Grapefruit in Florida. Plant Disease 80: 166-169.

Tucker, S.L., Talbot, N.J., 2001. Surface attachment and pre-penetration stage development by plant pathogenic fungi. Annual Review of Phytopathology 39: 385-417.

Van Zyl, J.G., Fourie, P.H., Schutte, C.G., 2013. Spray deposition assessment and benchmarks for control of Alternaria brown spot om mandarin leaves with copper oxychloride. Crop Protection 46: 80-87.

Van Zyl, J.G., Sieverding, E.G., Viljoen, D.J., Fourie, P.H. 2014. Evaluation of two organosilicone adjuvants at reduced foliar spray volumes in South African citrus orchards of different canopy densities. Crop Protection 64: 198-206.

van Zyl, S.A., Brink, J.C., Calitz, F.J., Coertze, S. and Fourie, P.H. 2010(a). The use of adjuvants to improve spray deposition and *Botrytis cinerea* control on Chardonnay grapevine leaves. Crop Protection 29: 58-67.

van Zyl, S.A., Brink, J.C., Calitz, F.J. and Fourie, P.H. 2010(b). Effects of adjuvants on deposition efficiency of fenhexamid sprays to Chardonnay grapevine foliage. Crop Protection 29: 843-852.

Vicent, A., Armengol, J., García-Jiménez, J., 2007. Rain Fastness and persistence of fungicides for control of Alternaria brown spot of citrus. Plant Disease 91: 393-399.

Vicent, A., Armengol, J., García-Jiménez, J., 2009. Protectant activity of reduced concentration copper sprays against Alternaria brown spot on 'Fortune' mandarin fruit in Spain. Crop Protection 28: 1-6.

Vicent, A., Armengol, J., Sales, R., García-Jiménez, J., Alfaro-Lassala, F., 2000. First report of Alternaria brown spot of citrus in Spain. Plant Disease 84: 1044.

Wagner, P., Fűrstner, R., Barthlott, W., Neinhuis, C., 2003. Quantitative assessment to the structural basis of water repellency in natural and technical surfaces. Journal of Experimental Botany 54: 1295-1303.

Western, N.M., Bieswal, M., Coupland, D., Hislop, E.C., Holloway, P.J., 1999. Drift reduction and droplet-size in sprays containing adjuvant oil emulsions. Pesticide Science 55: 633-675.

White, T.J., Bruns, T., Lee, S., Taylor, J.W., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pages 315-322 in: PCR Protocols: A Guide to Methods and Applications, eds. Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White, T.J. Academic Press, Inc., New York.

Whiteside, J.O., 1976. A newly recorded Alternaria-induced brown spot disease on Dancy tangerines in Florida. Plant Disease Reporter 60: 326-329.

Whiteside, J.O., Tmmer, L.W., 2000. Melanose. Page 28-29 in: Compendium of Citrus Diseases (second Edition). Timmer, L.W., Garnsey, S.M. and Graham, J.H. APS Press, The American Phytopathological Society, St. Paul Minnesota, USA.

Xu, L., Zhu, H., Ozkan, H.E., Bagley, W.E., Krause, R.C. 2011. Droplet evaporation and spread on hairly leaves associated with type and concetration of adjuvants. Pesticide Management Science 67: 842-851.

Zabkiewicz, J.A. 2007. Spray formulation efficacy-holistic and futuristic perspectives. Crop Protection 26: 312-319.

Zwertvaegher, I.K., Veraeghe, M., Brusselman, E., Verboven, P., Lebeau, F., Massinon, M., Nicolaï, B.M., Nuyttens, D. The impact and retention of spray droplets on a horizontal hydrophobic surface. Biosystems Engineering 126: 82-91.

Table 1. Properties of adjuvants evaluated.

Trade name	Registration holder	Main components	Properties	Chemistry classification	Active ingredient (g L^{-1})	Registered concentration (mL hL^{-1})
Break-Thru S240	Evonik Degussa Africa	Polyether-polymethylsiloxane-copolymer	Non-ionic-penetrant-surfactant	Organosilicone	1000	3
Citrole100	Total SA	Mineral oil	Mineral oil spreader/insecticide	Paraffinic oil complex	790	300
Entreé	Miller Chemicals SA	Emulsifiable vegetable oil	Non-ionic-wetter-spreader	Vegetable oil complex	818	60
Exit	Miller Chemicals SA	Emulsifiable vegetable oil	Non-ionic-wetter-spreader	Vegetable oil complex	819	250
Herbiplus	Total SA	Mineral oil	Mineral oil spreader/pH buffer	Paraffinic oil complex		300
Nu-Film-17	Miller Chemicals SA	Di-1-p-menthene	Non-ionic-sticker-spreader	Terpene oil	905	50
Villa51	Villa Crop Protection	Isotridecanol	Wetter-spreader	Alkylpolyethene glycol ether	918	18
Wetcit	Oro Agri SA	Borax and orange oil	Wetter-spreader-penetrant	Inorganic compound	10 and 50	100

Table 2. Deposition quantity (FPC%), deposition quality (ICD%), protected leaf area (%) and copper residue (mg kg^{-1}) as measured on 'Nova' mandarin leaves sprayed with copper oxychloride (2 g L^{-1}), fluorescent pigment (1 mL L^{-1}) and different adjuvants at pre- and post-run-off volumes of 1 and 3 mL per leaf, respectively.

Treatment[a]	Deposition quantity (FPC%)[b]		Deposition quality (ICD%)[b]		Protected leaf area (%)[b]		Cu residue (mg kg^{-1})[b]	
	Pre-run-off	Post-run-off	Pre-run-off	Post-run-off	Pre-run-off	Post-run-off	Pre-run-off	Post-run-off
Copper oxychloride	6.42 a	3.60 ab	21.82 d	53.94 a-d	74.86 a	61.11 abc	387 a	210 e
Nu-Film-17	6.70 a	4.13 ab	29.12 cd	33.50 bcd	64.95 ab	72.18 ab	189 f	237 d
Break-Thru S240	6.48 a	2.83 ab	27.73 cd	60.46 abc	63.02 abc	48.39 a-d	309 b	236d
Villa51	4.52 ab	1.67 ab	43.56 a-d	74.34 a	66.83 ab	22.56 bcd	125 j	99 l
Wetcit	3.61 ab	1.60 ab	49.65 a-d	67.75 ab	57.46 abc	17.07 cd	131 i	114 k
Citrole100	3.34 ab	1.80 ab	37.74 bcd	70.75 a	52.95 abc	31.51 a-d	263 c	236 d
Entrée	3.12 ab	0.96 b	58.27 abc	65.29 ab	25.60 bcd	5.35 d	165 g	135 h

[a]Refer to Table 1 for recommend adjuvant concentrations

[b]For each parameter separately, values in pre- and post-run-off columns followed by the same letter do not differ significantly ($P > 0.05$) according to Tukey's HSD test with a critical value of 4.993 for deposition quantity, quality and protected leaf area and 5.637 for Cu residue.

Table 3. Deposition quantity (FPC%), quality (ICD% and protected leaf area), and copper residue (mg kg^{-1}) determined on young 'Nova' leaves sprayed with different adjuvant treatments at recommended concentrations, yellow fluorescent pigment (1 mL L^{-1}) and copper oxychloride (2 g L^{-1}) to post-run-off and subsequently spray-inoculated with *Alternaria alternata* pv. *citri*.

	Deposition			Control		
Treatment[a]	**Quantity**	**Quality**		**Control**	**Predicted control**	**Cu residue**
	FPC%[b]	**ICD%[b]**	**Protected leaf area (%)[b]**	**(%)[b]**	**(FPC%)[bc]**	**(mg kg^{-1})[b]**
Copper oxychloride alone	5.02 a	48.12 c	61.51 a	54.82 a	78.82 a	106.42 bcd
Break-Thru S240	4.77 a	49.81 bc	62.97 a	64.19 a	77.32 a	153.64 abc
Nu-Film-17	4.60 a	48.17 c	62.38 a	64.16 a	74.72 ab	201.73 a
Citrole100	4.18 ab	62.36 a	51.99 ab	58.93 a	74.67 ab	179.75 ab
Villa51	3.06 abc	58.52 ab	44.49 b	60.18 a	61.26 bc	64.54 cde
Entreé	2.40 bcd	68.47 a	23.51 cd	73.13 a	54.29 c	65.59 cde
Herbiplus	2.20 cd	64.66 a	35.40 bc	61.53 a	51.52 c	
Wetcit	2.17 cd	58.58 ab	26.98 c	60.66 a	49.16 c	57.02 de
Exit	1.28 de	45.44 c	11.20 de	52.65 a	32.84 d	63.90 de
Water	0.00 e	0.00 d	0.00 e	0.00 b	0.00 e	8.87 e

[a] Refer to Table 1 for recommended adjuvant concentrations

[b] For each parameter separately, values in each column followed by the same letter do not differ significantly ($P > 0.05$) according to Tukey's HSD test with a critical value of 4.551.

[c] Predicted control calculated by subjecting deposition quantity data to FPC benchmark model (van Zyl *et al*. 2013): Control = $100*^{(1-e\ (-0.3346*\ \%\ FPC)}$

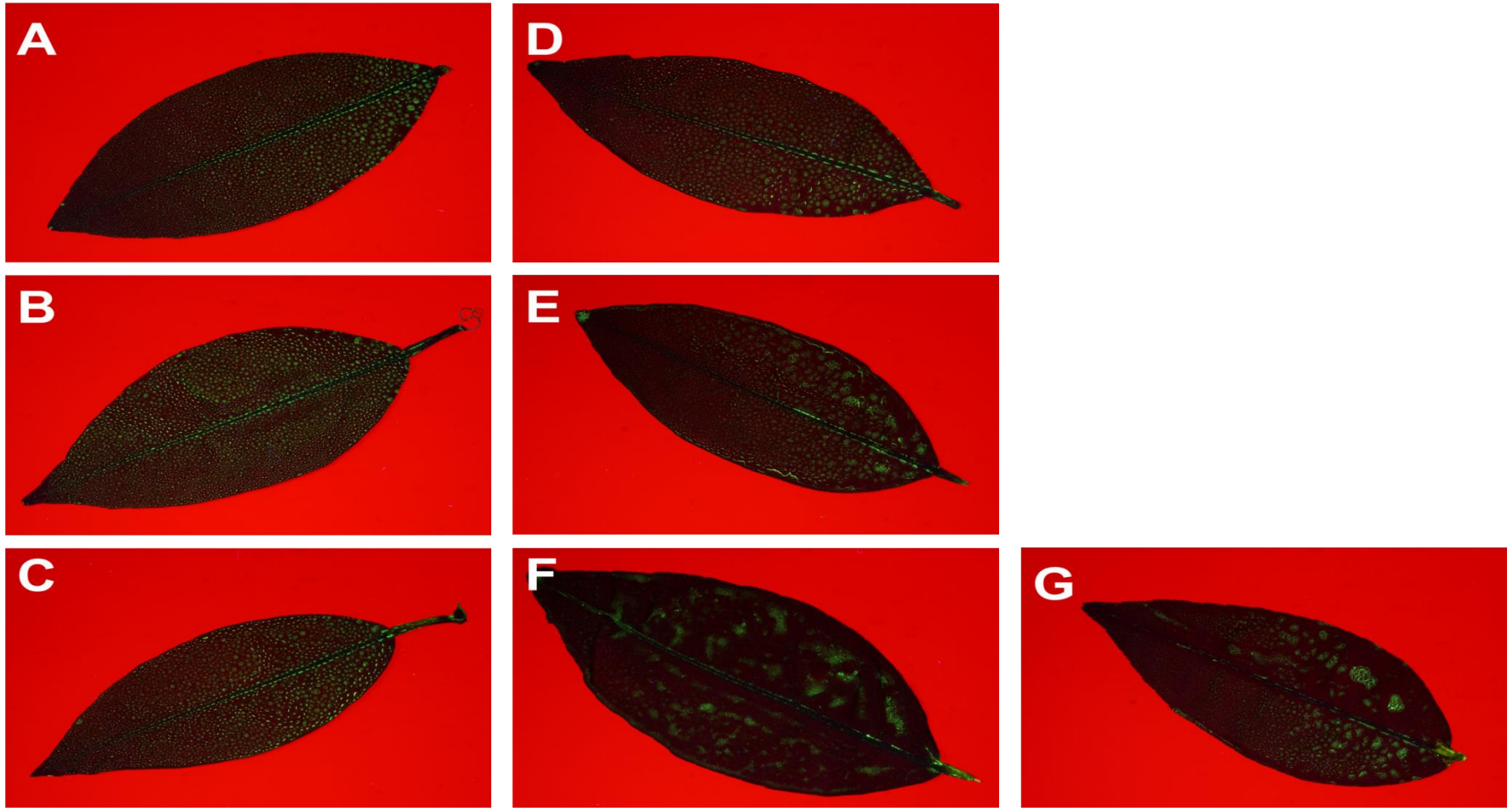

Figure 1. Digital images of the upper leaf surfaces of young 'Nova' leaves illuminated with UV-A light illustrating different deposition patterns retained when sprayed with different adjuvant treatments at pre-run-off application volumes (1 mL) with copper oxychloride alone (A), or the adjuvants Break-Thru S240 (B), Nu-Film-17 (C), Citrole100 (D), Villa51 (E), Entreé (F) or Wetcit (G) with copper oxychloride and SARDI Yellow Fluorescent Pigment.

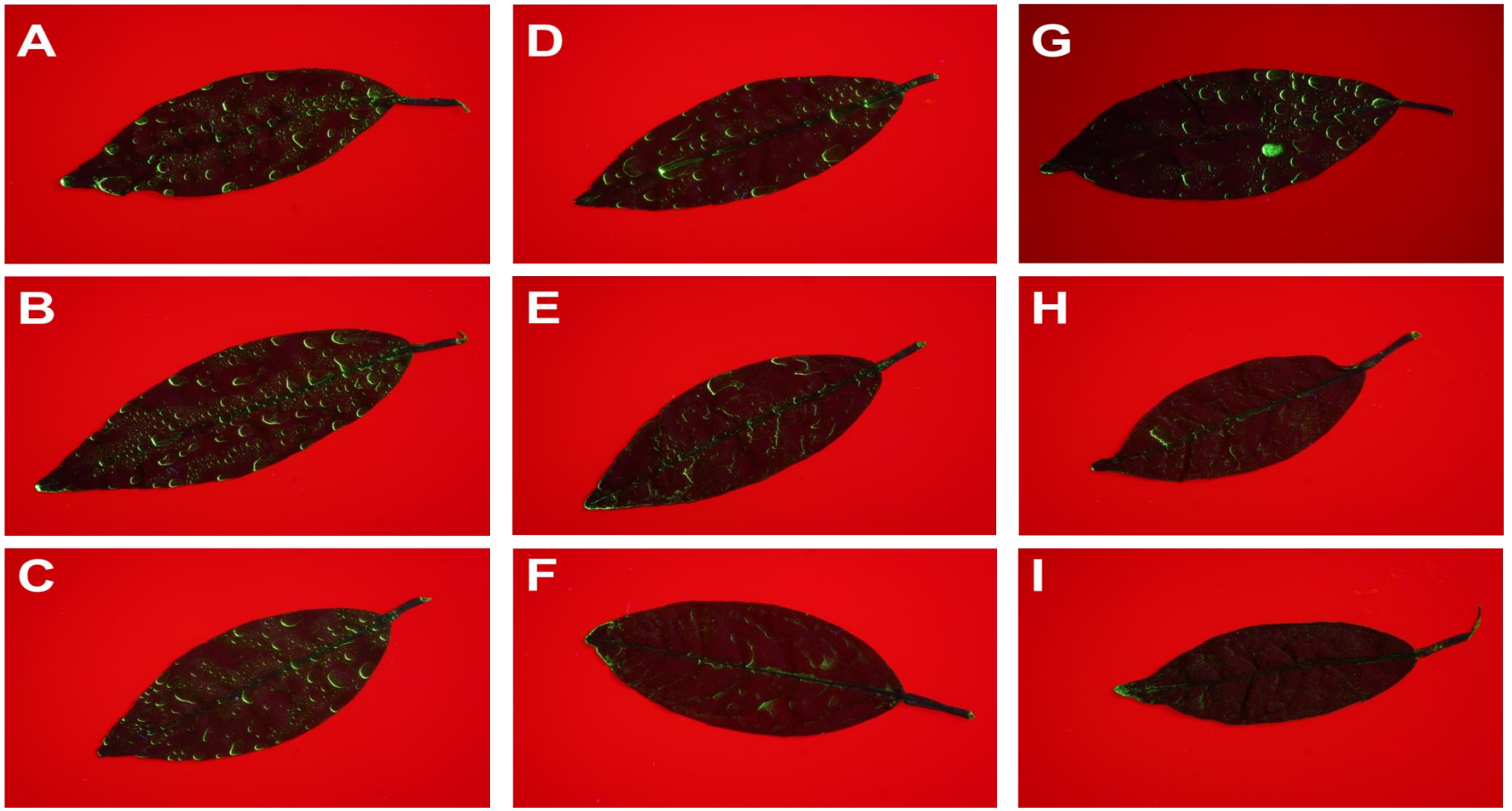

Figure 2. Digital images of the upper leaf surfaces of young 'Nova' leaves illuminated with UV-A light illustrating different deposition patterns attained when sprayed with different adjuvant treatments at post-run-off application volumes (3 ml) with copper oxychloride alone (A), or the adjuvants Break-Thru S240 (B), Nu-Film-17 (C), Citrole100 (D), Villa51 (E), Entreé (F), Herbiplus (G), Wetcit (H) or Exit (I) with copper oxychloride and SARDI Yellow Fluorescent Pigment

CHAPTER 6

Influence of selected adjuvants and copper oxychloride on the *in vitro* and *in vivo* development of *Alternaria alternata*

ABSTRACT

Spray adjuvants have the potential to improve deposition parameters and disease or pest control. However, previous research indicated that the addition of certain adjuvants to copper oxychloride (CuOCl) sprays on young 'flush' mandarin leaves for the control of Alternaria brown spot (ABS) resulted in poorer deposition quantity, but this did not result in reduced control of ABS compared with fungicide only sprays. In order to study this phenomenon, *in vivo* and *in vitro* studies were done to identify possible direct adjuvant effects on pathogen development and potential synergistic effects between the adjuvants and CuOCl. For the *in vivo* study, detached young 'Nova' mandarin leaves were sprayed with a post-run-off volume (3 mL) of selected adjuvants (Break-Thru S240, Nu-Film-17 and Entreé) alone and together with CuOCl at recommended concentrations. Leaves were left to dry and subsequently spray inoculated with *Alternaria alternata* (causal agent of ABS) and moist incubated for 6 h, whereafter 5-mm leaf discs were cut from leaves, placed on glass microscope slides and immediately stained with LIVE/DEAD BacLight staining solution. To determine the proportion of conidia that have adhered to leaf surfaces, another set of leaf discs were vortexed for 3 min before stained with the same solution. Using a microscope equipped with an epifluorescence condenser, viable conidia fluorescing bright green were counted on vortexed and non-vortexed leave discs and the percent adhered spores calculated. Additionally, germ tube growth, viability, appressoria formation and stress (visualized as percent red/orange fungal material *vs*. green fungal material per conidium or germ tube) were evaluated. Adjuvants alone did not influence conidial adhesion, appressorium formation, germ tube length and percent viable conidia compared with the water only control treatment. Adjuvant sprays together with CuOCl reduced conidial adhesion, germ tube length and percent viable conidia numerically; however, not significantly compared with CuOCl alone. Entreé realized conidial/germ tube stress similar to sprays in mixture with CuOCl and higher than found with the control treatment. For the *in vitro* study, a rapid multiplate microtiter protocol was optimized to use resazurin sodium salt (RZ) reduction/coulometric changes to evaluate the sensitivity of ABS to adjuvants alone and together with CuOCl. The amounts of CuOCl needed to inhibit 50% (EC_{50} – 35.50 mg L^{-1}) and 95% (EC_{95} – 281.66 mg L^{-1}) conidium germination and growth were determined by fitting CuOCl concentration and germination and growth inhibition data to a Michaelis-Menten growth model (R^2 = 0.774) realizing a very good correlation between observed and predicted values (r = 0.9909). Adjuvants alone did not inhibit germination or growth compared

with the water only control treatment. Adjuvants together with CuOCl improved germination or growth inhibition compared with the CuOCl treatment alone, although not significantly.

INTRODUCTION

Spray adjuvants are used regularly with foliar fungicide spray application to control diseases such as Alternaria brown spot (*Alternaria alternata* (Fr: Fr) Keissl., tangerine pathotype) and citrus black spot (CBS) (*Phyllosticta citricarpa* (McAlpine) van der Aa (syn. *Guignardia citricarpa* Kiely)) in citrus orchards. In the case of disease control, spray adjuvants are mainly used with the expectation that its addition will improve deposition parameters of contact fungicides and improve deposition and uptake of systemic fungicides. This in turn will improve preventative or curative efficacy of the fungicide used and improve control of the targeted disease.

In chapter 5, the addition of adjuvants to copper oxychloride (CuOCl) sprays to young mandarin leaves did not improve deposition quantity or quality of yellow fluorescent pigment compared to CuOCl alone sprays. In some cases, the addition of adjuvants resulted in poorer deposition parameters. However, the addition of adjuvants improved or realised similar control of Alternaria brown spot (ABS) than the CuOCl alone sprays. A relatively poor correlation between ABS control and CuOCl residue levels was evident and the poor correlation between ABS control and pigment deposition quantity further indicated that deposition quantity alone was not a reliable predictor of ABS control with CuOCl in combination with adjuvants. Furthermore, in chapter 5, poor correlations were found between copper residues and a yellow fluorescent pigment, used to measure deposition quantity. This is contradictory to previous studies describing the good correlation observed between pigment quantity and the contact fungicide residue (van Zyl *et al.*, 2013; Schutte *et al.*, 2012; Rossouw *et al.*, 2018), or pigment quantity and ABS control following sprays with CuOCl without the addition of adjuvants (van Zyl *et al.*, 2013). Van Zyl *et al.* (2013) developed a model that predicted the expected level of control of ABS based on the deposition quantity of yellow fluorescent pigment applied in combination with copper oxychloride. This model over- and under-predicted the levels of ABS control observed following sprays with adjuvants and CuOCl (Chapter 5). In some cases (eg. Entreé), deposition quantity and copper residues were significantly lower than the fungicide only control, but ABS control was superior. Alternatively, similar deposition quantities led to significantly higher copper residues, but similar levels of control. These anomalous results can possibly be ascribed to three reasons: (1) adjuvant effects on deposition parameters, especially deposition quality on a microscopic scale (Knoche *et al*., 1992; Gent *et al.*, 2003; Ryckaert *et al*., 2007); (2) direct adjuvant effects on pathogen development (van Zyl *et al*., 2010a, 2010b); and/or (3) synergistic effects between the adjuvant and fungicide (Orbovic, 2007).

Copper oxychloride's antifungal activity is on the surface of the plant as it has no known systemic action. CuOCl persists on the target surface after the spray has dried and does not redistribute as leaves and fruit expand (Whiteside, 1977; Albrigo *et al*., 1997; 2005; Vicent *et al*., 2007; Schutte *et al*., 2012). When the surface is rewetted, copper ions are gradually released from the copper residue on the host surface (McCallan, 1949; Richardson, 1997; RED, 2009), which provides the residual protectant activity against pathogens. Copper ions are absorbed by germinating fungal spores, which disrupts protein and enzyme function by binding to chemical groups such as the imidazoles, phosphates, sulfhydryls and hydroxyls. This action denature proteins and thereby inhibit enzyme production needed for cell function, which leads to sel damage and membrane leakage (Richardson, 1997; RED, 2009). Lahoz *et al.* (2017) evaluated the efficacy and persistence of CuOCl to control downy mildew when mixed together with an adjuvant derived from locust bean gum on grapevine leaves and bunches. Alone, the evaluated adjuvant did not have any effect on disease development, but it significantly increased persistence of CuOCl on leaves and bunches. The author ascribed this effect due improved deposition and distribution of copper particles by the adjuvant on target surfaces. Additionally, it was hypothesised that improved deposition and distribution of CuOCl on the target surface was the reason for improved disease control, since the likelihood of copper ions coming in contact with spores would be higher. Van Zyl *et al*. (2010a) evaluated the use of adjuvants to improve the deposition of fenhexamid on grapevine leaves for the control of *Botrytis cinerea*. Through fluorometry and microphotography, the author found that certain adjuvants improved *Botrytis* control and ascribed it to improved deposition quantity and quality. However, we observed a poor correlation between deposition quantity, quality and ABS control in our study (Chapter 5), where deposition quantity and quality were assessed at a macroscopic scale; for example, Entreé had poor deposition quantity and quality, but the best ABS control whilst Exit had the poorest deposition quantity but the best quality, and still realised similar ABS control than the CuOCl control treatment. Improved imaging sensitivity might address the limitations of assessing deposition quality on a macroscopic scale.

Published research on the physical, chemical or synergistic effects of adjuvants on the bio-efficacy of fungicides used in citrus is almost non-existing. Physical effects might be ascribed to the alteration of the plant cuticle by the adjuvant. The cuticle layer of each plant species is unique and it plays a major role in biotic interactions, like pathogen recognition (Craver and Gurr, 2006). Adjuvants can disturb the physical structure of the cuticle layer (Knoche *et al*., 1992; Zabkiewicz, 2007), apart from influencing the amount of active ingredient deposited and retained before and after spray run-off. Adjuvants are known to physically influence surface microstructures such as cuticular foldings and epicullicular waxes that minimise contact area between the spray droplet and the target surface (Wagner *et al*., 2003; Bargel *et al*., 2006) to increase deposition and/or retention (Hall *et al*., 1998). These physical

changes to the cuticle may also influence the ability of the pathogen to recognise the host and/or disrupt attachment (Tucker and Talbot, 2001; Carver and Gurr, 2006).

Recognition and adhesion of fungal spores to the plant surface is the first step in committing a pathogen to causing disease (Knogge, 1998). The environment the pathogen encounter on the plant surface is fundamental for host recognition, attachment and germination (Dean, 1997). Properties of the leaf surface such as the smoothness or roughness (Allen *et al.*, 1991) and the chemical composition (Dean, 1997) influence the ability of pathogens to adhere and grow. Wynn (1981) discussed how surface features influence germ tube tropism. The disturbance of epicuticular waxes may change the topography of the surface and through it, fungal tropism. With no recognition, spore adhesion, germination and appressorium formation is not possible (Tucker and Talbot, 2001). On the other hand, disruption of the cuticle layer might also improve pathogen development, as was observed by Knoche *et al*. (1992) and Rogiers *et al*. (2005).

Adjuvants can also have a synergistic or potentiating effect on the fungicide. For example, if an adjuvant reduces the pH of the CuOCl solution, the solubility of copper increases and so does the release of copper ions. Higher amounts of released copper ions can theoretically increase the efficacy against pathogen cells. Abbott (2016) found that certain adjuvants acidified the spray mixture, potentially improving the working of captan and reducing alkaline hydrolysis. Grayson *et al.* (1996a) evaluated the effect of adjuvants on the curative effect of dimethomorph in controlling downy mildew on grapevine leaves in a greenhouse study and found no fungicidal effect of an alcohol ethoxylate, an emulsifiable paraffinic oil and a vegetable oil adjuvant solution evaluated. However, disease control was improved when adding these adjuvants to dimethomorph sprays (Grayson *et al*., 1996a, 1996b). Dimethomorph has a translaminar systemic action and was applied in both studies as a curative spray.

Various methods exist to evaluate the sensitivity of pathogens to chemicals *in vitro* and *in vivo*. One method is through the histopathology study of the pathogen (fungal structures) microscopically on the leaf surface (*in vivo*) as influenced by fungicides. Van Zyl *et al.* (2010a) evaluated the use of adjuvants alone and together with fenhexamid for the control of *Botrytis cinerea* on grapevine leaves. This was accomplished through studying deposition parameters microscopically and the effect it had on *Botrytis* by means epifluorescence microscopy. Adjuvants alone did not reduce germination, but increased spore mortality or germ tube growth compared with the water only control treatment. The addition of certain adjuvants to fenhexamid sprays reduced germination and germ tube growth compared with the fenhexamid only spray. The authors ascribed this to improvement in deposition and possibly direct or indirect effects of the adjuvant on pathogen development.

In vitro assays have been successfully used to evaluate sensitivity of ABS-causing isolates of *A. alternata* to iprodione (Solel *et al*., 1996) and azoxystrobin, pyraclostrobin and fenbuconazole (Mondal *et al*., 2005). Vega *et al.* (2012) developed a rapid resazurin-based microtiter assay to evaluate the sensitivity of *A. alternata* isolates to quinone outside inhibitor (QoI) fungicides. The assay consists of using resazurin redox die. Resazurin (RZ) is a tetrazolium-based die that is stable, water soluble and non-toxic. It is blue and nonfluorescent in its oxidised form (To *et al.*, 1995). When reduced through cell metabolism, the blue colour is reduced to a purple-pink colour, which is fluorescent. The process of reduction happens in the final stage of O_2 reduction in the cytochrome oxidase region. The colour reduction can be measured fluorometrically or spectrophotometrically (Kalina and Palmer, 1968). The microtiter assay developed by Vega *et al.* (2012) used reduction of RZ through conidial germination and proved successful and sensitive enough to determine QoI-fungicide sensitivity in *A. alternata.* The aim of this study was to microscopically study possible direct effects of selected adjuvants alone and together with CuOCl on *A. alternata* development on the leaf surface, and to determine the sensitivity of *A. alternata* to adjuvants alone or in combination with CuOCl using a RZ-based microtiter assay.

MATERIALS AND METHODS

Spore adhesion and growth as influenced by adjuvants

Leaves

ABS-susceptible ‘Nova’ mandarin hybrid (*Citrus reticulata* Blanco; hybrid of Clementine ‘Fina’ and tangelo ‘Orlando’) trees were grown in 10-L plastic pots in a glasshouse at 27°C. Drip irrigation and a monthly application of slow release fertilizer (3:1:2 of N:P:K) were used to maintain the plants. The trees were regularly pruned to stimulate young growth (flush) production for use in experiments and too keep the trees small.

Inoculum

An isolate of *A. alternata* was obtained from symptomatic mandarin leaves with typical ABS symptoms. It was single-spored and thereafter identified using conidium morphology as *A. alternata*. Pathogenicity tests on susceptible ‘Nova’ mandarin leaves confirmed it to be the tangerine pathotype of *A. alternata* (Whiteside, 1976). It is stored in the Stellenbosch University culture collection (STE-U no. 6593). Single spore isolates were placed on potato dextrose agar (PDA; MERCK Biolab, Gauteng, South Africa) plates and incubated at 27°C for 7 to 14 days under 12-h light-dark cycle until abundant conidia were observed. Conidial suspensions were produced by pouring sterile water onto the PDA cultures and rubbing the surface gently with an L-shaped glass rod. The conidial suspension was filtered through two layers of cheesecloth and adjusted to 1×10^5 conidia mL L^{-1} with the use of a haemocytometer.

To prevent loss of fitness of the isolate, it was regularly inoculated and re-isolated from non-treated 'Nova' mandarin leaves.

Spray application

Five young (flush) shoots were cut from 'Nova' mandarin trees in the glasshouse. Upper leaf surfaces of fresh detached young 'Nova' leaves (smallest: 2 to 3 days old, ± 15×8 mm; largest: 7 to 10 days old, 55×30 mm) were sprayed by means of a gravity feed mist spray gun (ITW DEVILBISS Spray Equipment Products, USA) with a fluid nozzle tip of 1.5 mm in diameter mounted on a spray frame (steel framework 800×1410×660 mm). A single leaf was positioned on a wire mesh tray (angled at 30° to the bench top), while the spray gun was mounted at a distance of 600 mm away aiming squarely at the target. A post-run-off volume (3 mL) of selected spray adjuvants in deionised water at recommended concentrations were sprayed alone and together with a contact copper oxychloride fungicide [Villa Copper Oxychloride, 85% WP (Villa Crop Protection SA, Kempton Park, South Africa); with 50% metallic copper equivalent; 2 g L^{-1})] on each leaf. Based on the results obtained in chapter 5, three adjuvants were selected for further evaluation: Break-Thru S240 [non-ionic surfactant super spreader/penetrant; 75% modified polyether modified trisiloxane (Evonik Degussa Africa, Midrand, South Africa); 0.03 mL L^{-1}], Nu-Film-17 [Non-ionic sticker-spreader; 90.6% di-1-p menthene (Hygrotech, Pretoria, South Africa); 0.5 mL L^{-1}] and Entreé [Non-ionic activator/enhancer; 81.9% vegetable oil (Miller Chemicals, South Africa); 0.6 mL L^{-1}]. Deionised water alone was sprayed on upper leaf surfaces as a negative control treatment, whilst CuOCl alone was sprayed to serve as a positive control. Spray application was done at a constant pressure of 185 kPa from an air compressor (Balma® 50 L; 1.5 kW; www.balma.com), custom-fitted with activated carbon filters to remove any possible oil contamination. The spray gun was cleaned with 70% ethanol solution, flushed with distilled water and air-dried after each treatment. The wire mesh trays were cleaned with 70% ethanol solution between treatments. Five leaves were sprayed separately per treatment combination as experimental units. Spray treatments were staggered at 45-min intervals to allow adequate time for subsequent treatments. Treated leaves were carefully hung to air dry after each spray. After leaves had dried (1-2 h), they were carefully placed with unsprayed side facing down, on water-soaked paper towels inside plastic containers (300×60×250 mm) for inoculation.

Inoculation

Upper leaf surfaces of treated leaves were spray inoculated with a pre-run-off volume (0.5 mL) of 1 × 10^5 spores mL^{-1} suspension *A. alternata*. Spray inoculation was done in the same manner described for spray application. The spray inoculated leaves were placed on moistened paper towels and incubated in the plastic containers at high relative humidity

(>95%) at 27°C in the dark for 6 h. Longer incubation times made counting and measuring individual conidia and germ tubes difficult due to dense germ tube growth over the leaf disk complicating visualisation of individual conidia and germ tubes. Inoculation treatments was staggered by 45 min to allow sufficient time for staining and leaf surface microscopic observation and measurements, as described below.

Evaluation of conidial adhesion

After the 6-h incubation period, one treatment at a time, leaves were removed from the moisture chamber. Of the five sprayed leaves, three were randomly selected for evaluation. Two leaf disks were cut from each of the selected leaves with a 5-mm diameter sterilised cork borer, one on either side of the leaf midrib (six disks per treatment). Three disks were placed on separate glass slides and stained immediately in the dark by staining the leaf disc with 30 µL LIVE/DEAD® *Bac*Light staining solution (www.lifetechnologies.com) (Chang and Séguin-Swartz, 2002) for 5 min. After the staining period, a glass cover slip was placed on the leaf disk. The other three leaf disks were placed into individual 1.5 mL Eppendorph tubes filled with 1 mL dH2O. The tube was subsequently vortexed for 3 min. After this process, the leaf disk was carefully removed with a tweezer, care being taken not to disturb the surface of the leaf disk and placed on clearly marked glass slides. The same staining procedure was subsequently applied to the vortexed leaf disks after which it was covered by glass cover slips. The staining solution was kept on ice and in the dark throughout the process.

After staining, conidial adhesion as influenced by adjuvants with and without copper oxychloride, was evaluated by counting all conidia on the control and vortexed leaf disk surfaces (at least 50) using a Zeiss Axioskop microscope at ×200 magnification, equipped with an epifluorescence condenser, a high-pressure mercury lamp using Zeiss 02 and 06 Neofluar objectives with a G365 excitation filter (www.zeiss.com). With this setup, germinated conidia fluoresced bright green (viable) and different shades of orange to red (stressed or dead, respectively) (Chang and Séguin-Swartz, 2002). The percent adhered spores were subsequently calculated as ((vortexed/control) × 100). The experiment was repeated five times.

Germ tube growth, appressorium formation and spore viability

This experiment was conducted using the same methodology as to determine spore adhesion and germination. For this study, spray and inoculation treatments were staggered 30 min apart to allow for sufficient staining and digital photo capturing time. Leaf disks were not vortexed. Ten digital photos (*.TIFF format; 4116×3072 pixels; 35-40 MB) were taken at ×200 magnification spaced in a ‘Z’ pattern across a stained leaf disk (three leaf disks per treatment) with a Nikon DMX 1200 microscope camera (www.nikon.com) mounted on a Zeiss Axioskop

(www.zeiss.com). The following parameters were evaluated on the ten digital photos per stained leaf disk using Image Pro Plus 7.0 (www.mediacybernetics.com):

(1) Conidium viability: conidia were classified as 'viable' if the conidia fluoresced bright green or orange and/or had one or more germ tube that was longer than the diameter of the conidia, 'inhibited' if it fluoresced red or not at all (dark black/brown) with no germ tube growth after 6h. Colour discernment was done using the Count/Size command manually on RGB (Red Blue Green) channel of digital images. The following histogram values were used: Red: 128,0,0 to 255,40,60; Orange: 255,69,0 to 255,215,0; Green-blue: 154,205,50 to 0,255,127. The whole image was used as an Area of Interest (AOI). Using the Data Collector function, the number of viable conidia (green/orange) and inhibited conidia (red/brown/black) was collected. Each digital photo was manually checked if all conidia were identified. The number of viable conidia were divided by the total amount of conidia evaluated per slide (total of ten digital photos) and was expressed as percent viable conidia (%).

(2) Germ tube length (µm): viable conidia that produced germ tubes were measured. Measurement was made from conidial germination point to the tube's growth apex, for each germ tube separately using the manual measurement "trace" function. To ensure accurate measurements, software was calibrated using the Spatial Calibration function for each trial. The calibration was done on a scale (µm) inserted onto a reference image (normal leaf surface image with scale) using NIKON Control software ACT 1 (ww.nikon.com) at 200× magnification. Using the Data Collector function, the number and length (µm) of germ tubes per conidia was recorded. For statistical analysis, the sum of germ tube lengths per conidium was used.

(3) Conidium or germ tube stress was visually assessed qualitatively as the percent red/orange fungal material vs. green fungal material per conidium and/or germ tube (%). The following qualitative scale was used per conidia/germ tube in 10% increments: lower limit of the scale was 0% of fungal material fluorescing red/orange and 100% green-blue (0% stress), while the upper limit of the scale was when 100% of fungal material fluoresced red/orange and 0% green-blue (100% stress). The higher the proportion of fungal material fluorescing red/orange over material fluorescing blue/green, a higher percent stress was assigned (Figure 1).

(4) Appressorium formation: appressorium formation was identified as a swelling at the end of the germ tube that was larger than double the diameter (µm) of the germ tube and was expressed as the average number of appressoria formed per conidium (Figure 2).

All measurements and counts were made using digital image analysis software Image Pro Plus 7.0. The experiment was repeated three times evaluating 1288 conidia in total.

Sensitivity to adjuvants and copper oxychloride

Optimisation of microtiter assay

One isolate of *A. alternata* was evaluated (STE-U no. 6593). The optimal resazurin (RZ) concentration, conidial concentration and incubation time was evaluated as follows: 14- to 21-day-old conidia were harvested using the same methodology as previously described and spore suspensions adjusted to 5×10^3, 1×10^4, 5×10^4 and 1×10^5 conidia per mL using a haemocytometer. One gram RZ sodium salt (Sigma-Aldrich, Steinheim, Germany) was dissolved in 100 mL of dH_2O to make a 40 mM stock solution. From the stock solution, a dilution series was made to 300, 400, 500 and 600 µM RZ at a pH of 6.5. RZ media selection was based on media evaluated by Vega *et al.* (2012), which found that complete media (CM) (Bennett and Lasure, 1991) realised the most consistent RZ reduction over time for *A. alternata.* For each RZ × conidial concentration combination, 100 µL of CM was added to the wells of a 96-well, flat bottom crystal clear microplate (Greiner Bio-One GmbH, Frickenhausen, Germany) in quadruplet. Eighty microliters of conidial concentrations and 20 µL of RZ concentrations were added to two of the wells for each treatment combination. This realised a total well volume of 200 µL and RZ concentrations of 30, 40, 50 and 60 µM. One of the four wells was loaded with 100 µL dH_2O to serve as a blank (for background subtraction), whilst the remaining well was loaded with 80 µL dH2O and 20 µL RZ per treatment combination. In total, 64 wells per plate were used. Optimal incubation time was evaluated by loading one microplate for each incubation time selected (12, 14, 16, 18, 20, 22 and 24 h). All plates were loaded in the dark in an aseptic manner in a laminar flow cabinet. Loaded plates were sealed separately in black sterile plastic bags and incubated at 27°C and 150 rpm for the specified incubation times (12 to 24 h) in an incubator-shaker. Plate specific absorbance was measured at 570 and 600 nm with a microplate reader (FLUOstar OPTIMA, BMG LABTECH GmbH, Ortenberg, Germany) after each incubation time. A microplate was only opened and measured once to keep the protocol aseptic. A colorimetric assay was used as an indicator of metabolic or respiratory activity of fungal spores and was measured as percent RZ reduction (%). Percent RZ reduction was calculated for each well in each experiment using the following formula as derived from instruction sheets as used by Vega *et al*. (2012):

$$\frac{(\varepsilon_{ox})\lambda_2 A\lambda_1 - (\varepsilon_{ox})\lambda_1 A\lambda_2}{(\varepsilon_{red})\lambda_1 A'\lambda_2 - (\varepsilon_{red})\lambda_2 A'\lambda_1} \times 100$$

Where:

(ε_{ox}) = molar extinction coefficient of RZ oxidised from blue: 570 nm = 80 586 and 600 nm = 117 216

(ε_{red}) = molar extinction coefficient of RZ reduced from pink: 570 nm = 155 667 and 600 nm = 14 652

A = absorbance of test well – background well

A' = absorbance of negative control well – background well

λ_1 = 570 nm

λ_2 = 600 nm

The experiment was repeated four times.

Copper oxychloride sensitivity

Alternaria alternata sensitivity to a copper oxychloride fungicide (Demildex, copper oxychloride 85% WP, Delta Chemicals, Meyerton, South Africa; with 50% metallic copper equivalent; 2 g L^{-1}) (CuOCl) was evaluated. A stock solution of 10 000 mg L^{-1} was prepared for the CuOCl in 0.5 L distilled H_2O. The stock solution was not filter sterilised as done by Rampersad (2011), due to the particle size distribution of the copper formulation not allowing it. Complete Media (CM) (125 mL) was amended with a CuOCl concentration ranging from 0 (control) 2, 10, 20, 30, 40, 100, 140, 200, 300, 400, 500, 600, 700 mg L^{-1}, of which 100 µL of each CM amended concentration range was loaded in six separate test wells of the same type of microplate as previously used. *A. alternata* (80 µL of 1×10^5 conidia per mL suspension) and 20 µL of a resazurin-based (RZ) *in vitro* toxicology assay kit (Sigma-Aldrich, Steinheim, Germany) was added to four of the six test wells per CM amended concentration. The RZ kit was used instead of the RZ salt solution since it was found to be easier to use (less laborious), more light stable (less prone to bleaching), realizing more consistent reduction results and with similar RZ reduction percentages over 24 h as determined in the optimisation trials. For each amended CM concentration, one of the six wells was loaded with 100 µL distilled H_2O to serve as a blank (background subtraction), whilst the remaining well was loaded with 80 µL dH2O and 20 µL of RZ kit. This realised a final concentration range of half of the amended CM media range (0, 1, 5, 10, 15, 20, 50, 70, 100, 150, 200, 250, 300, 350 mg L^{-1}). All plates were loaded and sealed as described previously. Plates were incubated at 27°C and 150 rpm for 24 h in an incubator as determined previously.

RZ absorbance and RZ reduction percentage was determined and calculated as described previously. Germination/growth inhibition was calculated from percent RZ reduction by first calculating the relative germination/growth percent ((CuOCl amended well) / control well × 100)) and then percent germination/growth inhibition (100 – relative germination/growth). CuOCl concentration and percent germination/growth inhibition data were plotted on various non-linear regression models to find the best fit. The trial was repeated four times.

Adjuvant sensitivity

The sensitivity of *A. alternata* to adjuvants used regularly with fungicides were evaluated. These adjuvants were Break-Thru S240, Nu-Film-17, Citrole100 [Mineral oil; 3 mL L^{-1} (Total South Africa)], Entreé, Wetcit [Surfactant; 1% Borax; 5% Orange oil; 1 mL L^{-1} (Oro Agri South Africa)] and Villa51 [Surfactant; 91.8% isotridecanol; 0.18 mL L^{-1} (Villa Crop Protection, South Africa)] (as used in previous chapters). Complete Media (CM) (125 mL) was amended with adjuvants alone and combined with double the determined EC_{50} concentration (35.50 mg L^{-1}) of copper oxychloride (CuOCl) to realize the EC_{50} concentration in the test wells after addition of the spore suspension and RZ solution. For CuOCl and adjuvant treatments, 10 000 mg L^{-1} stock solutions were prepared in dH_2O. Adjuvant concentrations used were calculated as the recommended concentration in proportion to that of the CuOCl registered concentration for field application (2 g L^{-1}). This was 1.5% for Break-Thru S240, 25% for Nu-Film-17, 30% for Entreé, 150% for Citrole100, 50% for Wetcit and 9% for Villa51. As with CuOCl, the adjuvant concentrations were doubled to realise the recommended (1×) concentrations in the wells. The pH of each of the solutions were measured with a calibrated Jenway 3310 pH meter (www.jenway.com). One hundred microliters of each CM amended with adjuvant alone or with adjuvant and CuOCl was loaded in six separate test wells. As previously optimised, 80 µL of 1×10^5 conidia per mL *A. alternata* and 20 µL of RZ-based *in vitro* toxicology assay kit was added to four of each treatments' test wells. Plates were loaded and incubated for 24 h as previously described. Germination/growth inhibition (GGInhibition) was calculated from percent RZ reduction as described previously. The trial was repeated 3 times.

Statistical analysis

A randomised block design was used for experimental layout for evaluating spore adhesion and growth as influenced by adjuvants. Percent adhered conidia (%), total germ tube length (µm), percent viable conidia (%) and percent conidia/germ tube stress (%) and average number of appressoria formed per conidia data were subjected to analysis of variance (ANOVA). Percent RZ reduction optimisation data based on conidia and RZ concentration data over time was also subjected to ANOVA. Tukey's honest significant difference (HSD) test was used to identify significant differences between treatments at a 95% confidence interval. For the CuOCl sensitivity assays, germination/growth inhibition% data were subjected to multiple linear and non-linear regression models to determine best fit. The model that fitted best was used to determine the EC_{50} and EC_{95} of CuOCl. In turn, *A. alternata* germination/growth inhibition as influenced by adjuvants alone and together with CuOCl was subjected to appropriate ANOVA and Tukey's HSD test at a 95% confidence interval. All

statistical analysis was done using SAS statistical software (Version 9.2; SAS Institute Inc., Cary USA) (www.sas.com) or XLSTAT version 19.03 (www.xlstat.com).

RESULTS

Spore adhesion and growth as influenced by adjuvants

Conidial adhesion

Analysis of variance of percent adhered conidia (%) indicated significant effects for spray treatment ($P < 0.0001$). None of the adjuvants alone (82.90% to 91.27%) reduced adhesion significantly in relation to the water only treatment (83.50%). The addition of CuOCl to the adjuvants sprays had a significant effect, with the percent conidial adhesion reducing dramatically. Entreé (37.81%) and Break-Thru S240 (53.19%) sprayed together with CuOCl did not differ from the CuOCl alone spray (41.95%) with Nu-Film-17 + CuOCl realising significantly higher levels of adhesion (61.02%) (Table 1).

Germ tube length

Analysis of variance of germ tube length (µm) indicated significant effects for spray treatment ($P > 0.0001$). Germ tube length on leaf disks sprayed with adjuvants alone (117.08 to 78.16 µm) did not differ from the water only control treatment (90.56 µm) after 6 h incubation. The addition of CuOCl to adjuvant sprays reduced germ tube length significantly. Similar lengths were measured on leaf disks sprayed with CuOCl and adjuvants (13.11 to 22.05 µm) compared with CuOCl alone (21.10 µm) (Table 1).

Conidium viability

Analysis of variance of percent viable conidia (%) indicated a significant main effect for treatment ($P < 0.0001$). The highest percentage spore viability was realised on leaf surfaces sprayed with adjuvant treatments (99.17 to 95.71%) and the water only control treatment (92.45%). The addition of CuOCl to sprays decreased viability significantly. Similar viability was found with CuOCl sprays alone (32.84%) and together with adjuvants (31.99 to 46.93%) (Table 1).

Conidium and germ tube stress

Analysis of variance of conidium and germ tube stress (%) indicated a significant main effect for treatments applied ($P = 0.016$). Conidia and germ tubes on leaves sprayed with Break-Thru S240 (85.27%) and Nu-Film-17 (72.83%) alone were similarly stressed than the control (water only) treatment (65.17%). Interestingly, Entreé realised similar high stress (94.70%) to that realised by the adjuvants + CuOCl, significantly higher than the control treatment (Table 1).

Appressoria formed

Analysis of variance of the number of appressoria formed per conidium indicated a significant effect for treatments (P = 0.022). Multiple comparison evaluation indicated no differences among treatments. Adjuvant sprays alone reduced the formation appressoria in relation to the water only control treatment, although not significantly. An average of 1.61 appressoria was formed per conidium in the water only control treatment. Break-Thru S240 formed an average of 1.25 appressoria; 22% less appressoria than the control treatment. Nu-Film-17 (0.49 per conidia) and Entreé (0.36 per conidia) reduced appressorium formation by 69.6 and 77.6%, respectively, compared with the control treatment. The addition of CuOCl to adjuvant treatments reduced appressoria formation by 94 to 98% (0.031 to 0.092 appressoria per conidium); however, not significantly compared with the water only control treatment (Table 1).

Sensitivity to adjuvants and copper oxychloride

Optimisation of microtiter assay

Analysis of variance of RZ reduction (%) indicated significant interactions for time × conidium concentration (P < 0.0001) and time × RZ concentration (P < 0.0001). As incubation time and conidium concentration increased, so did the RZ reduction. At 12- and 14-h incubation, the lowest RZ reduction was observed, with RZ reduction being similar for 5×10^3 to 1×10^5 conidia per mL (9.15 to 22.25 %). At 16 h, 1×10^5 conidia per mL realised significantly higher RZ reduction than all lower conidial concentrations (30.68 *vs.* 21.77 to 14.18%). This was also observed at 18 h (37.23 *vs.* 14.18 to 26.13%), 20 h (51.20 *vs.* 17.51 to 35.01%) 22 h (68.25 *vs.* 19.82 to 47.94%) and most prominently, 24 h incubation. The highest reduction of RZ was realised with a conidium concentration of 1×10^5 at 24 h, significantly more than all other incubation times and conidium concentration combinations (89.06%) (Figure 3).

For all incubation times separately, RZ reduction did not differ between RZ concentrations (results not shown). The highest percent RZ reduction was found to be after 24 h at 30 µM (56.75%). This was significantly higher than realised at 12 to 22 h incubation for all RZ concentrations (44.45 to 9.67 µM) (Figure 3).

Copper oxychloride sensitivity

A blue to pink colour change similar to that observed in the optimisation trials was observed in the test wells after 24 h incubation. As the CuOCl concentration in the wells increased, the colour change from blue to pink decreased, due to decreased RZ reduction. The concentrations of CuOCl (mg mL^{-1}) needed to inhibit 50% (EC_{50}) and 95% (EC_{95}) conidium

germination and growth were determined from fitting the data to a Michaelis-Menten growth model, which fitted the data best (R^2 = 0.7748); Figure 4):

$$Y = \frac{\alpha X}{\beta + X}$$

Where Y = Germination / Growth Inhibition (%);

X = CuOCl concentration in mg L^{-1}

α = 109.164

β = 41.9947

A good correlation was realised between observed and predicted values (r = 0.9909). EC_{50} and EC_{95} values were calculated to be 35.50 and 281.66 mg L^{-1} CuOCl, respectively.

Adjuvant sensitivity

Analysis of variance of pH and germination/growth inhibition data indicated a significant effect for treatment (P < 0.0001). The pH measured for Entreé (6.65) was significantly higher than all other treatments, with the lowest pH realised by CuOCl alone (6.20), significantly differing from all treatments except Citrole100 (6.27). The rest of the treatments realised pH between 6.65 and 6.20 (Table 2). No correlation could be found between pH and germination/growth inhibition. Over the 4 trials, germination/growth inhibition of adjuvant treatments alone (1.54 to 4.61%) did not differ significantly from the control treatment (0%). Copper oxychloride had a significant effect on inhibition (49.14%). Adjuvants with CuOCl all realised similar (53.31 to 59.60%) germination/growth inhibition than the CuOCl alone treatment (Table 2).

DISCUSSION

Through an *in vivo* and an *in vitro* study, results from this chapter indicated that the adjuvants evaluated generally did not influence the germination or development of *Alternaria alternata* at statistically significant levels. However, some level of stress was exerted on the germinating conidia, which resulted in fewer appressoria forming in the adjuvant treatments. CuOCl was shown to be an effective germination inhibitor and that the addition of adjuvants did not have a synergistic influence on the effectiveness of the fungicide. Additionally, the study confirmed the suitability of the rapid multiplate microtiter protocol using resazurin sodium salt (RZ) to evaluate the sensitivity of *A. alternata* to contact fungicides (Vega *et al.,* 2012).

The *in vivo* and *in vitro* study was done in an attempt to help explain the results obtained in chapter 5, *i.e.* where the addition of adjuvants to CuOCl sprays improved or realised similar control of ABS as the CuOCl alone spray, despite the observations that some of these adjuvants resulted in poorer spray deposition parameters and reduced copper residues on leaf surfaces.

The first hypothesis investigated to explain these anomalies was that the addition of certain adjuvants physically altered the cuticle layer of the leaf surface, thus possibly influencing recognition, adhesion and penetration of *A. alternata* conidia. An attempt was initially made to use scanning electron- and confocal microscopy to study the leaf surface and pathogen reaction. However, electron bombardment in scanning electron microscopy was too harsh on the young leaf surface (3 to 10 days old), destroying the leaf when not fixated for viewing. Fixation of the leaf sample through standard protocols such as gold fixation or more advanced methods such as critical point (CP)-drying, glycerol substitution (Peacock *et al*., 1998; Ensikat *et al*., 2010) and freeze dry methods (Njombolwana *et al.*, 2013) were also not viable since the process destroyed the leaf; young "flush" mandarin leaves were just too sensitive. Older leaves could not be used since mature leaves are moderate to highly resistant to infection (Solel and Kimchi, 1998). Future studies should focus on fixation methods sensitive enough to enable viewing of young sensitive leaf surfaces. Confocal microscopy was not sensitive enough to visualise and measure physical changes to the leaf surface (cuticle layer) and too laborious and time consuming for high-throughput histopathology study.

Use of the Zeiss Axioskop microscope at ×200 magnification, equipped with an epifluorescence condenser to evaluate conidium adhesion, germ tube growth, appressorium formation and viability as influenced by adjuvants alone and together with CuOCl proved to be successful and informative. The use of LIVE/DEAD® *Bac*Light staining solution (Chang and Séguin-Swartz, 2002) made visualisation of conidia, germ tubes and appressoria on the leaf surfaces possible with conidia fluorescing bright green (alive) and orange to dark red (stressed or dead, respectively) easy to differentiate. After the 6-h incubation period, attached conidia were producing one or more germ tubes, depending on the size of the conidia. Germ tubes grew mostly randomly on the surface, with some growth in the direction of nearby stomata. This concurred with the findings of Solel and Kimchi (1998). Stomata were smaller, few and far apart on the upper leaf surface compared with a relative abundance on bottom leaf surfaces of the young mandarin leaves. Appressoria were observed as oblate swelling of the germ tube apex. Six hours of incubation was an optimal time for observation since conidia were attached, germ tubes growing and appressoria developing. Observation after longer incubation times made observation of fungal structures difficult due to germ tubes overgrowing each other and becoming too dense.

Vortexing of leaf surfaces after the incubation period proved to be successful in removing any conidia that have not attached, thereby allowing us to study the influence of treatments on the attachment of *A. alternata* conidia. Microphotography of leaf surfaces made staggering of photo capturing intervals much easier to ensure all treatments were viewed at similar incubation time. The use of image analysis software Image Pro Plus 7.0 made measuring of germ tubes and counting of appressoria easy, fast and accurate.

Recognition and attachment are important processes as precursors for penetration or invasion of the host during the disease cycle (Mendgen, 1996; Knogge, 1998; Epstein and Nicholson, 1997; Agrios, 2005). The young mandarin leaves used in this study can be regarded as easy to moderately wettable with a relatively smooth and thin cuticular surface and newly developed wax layer (Baker *et al*., 1975). Thus, one can argue that adjuvants, depending on chemical composition, can disturb the still developing wax layer (Kirkwood, 1999; Hess and Foy, 2000). However, none of the adjuvants influenced attachment significantly. Santier and Chamel (1996) found vegetable oils, such as Entreé, increase the uptake/absorption of herbicides by decreasing the viscosity of the wax layer and by disturbing its crystalline structure. Kirkwood (1999) found other non-ionic formulations, depending on lipophilic/hydrophilic balances, can plasticise or elasticise the lipid components in the wax layer, or cause hydration of polar compounds in the wax layer. After attachment and germination, *A. alternata* can start producing ACT-toxin before direct penetration or invasion. Since toxin production is host specific, host recognition must have occurred before or during the attachment phase (Solel and Kimchi, 1998). Thus, if the adjuvants had any effect on the surface structure of the leaf, as found by Santier and Chamel (1996) and Kirkwood (1999), that would disrupt disease development and host recognition, the above-mentioned evaluations would indicate it. No significant adjuvant-alone treatment effect was observed for conidium adhesion, viability or germ tube length. Entreé alone, however, increased the percent stress significantly by 45.3% compared with that of the control treatment and at similar levels to that of the CuOCl alone treatment. For conidium adhesion, some notable adjuvant effects were observed in combination with CuOCl: significantly more conidia adhered to the surface when CuOCl was applied with Nu-Film-17 (45.5% more adhered conidia than CuOCl alone), with Break-Thru S240 + CuOCl also resulting in 26.8% more adhered conidia than the CuOCl spray alone. Conidium / germ tube stress was also marginally higher. These effects on conidium / germ tube stress and adhesion should be studied further in the future.

Nu-Film-17 and Entreé sprays alone reduced appressorium formation by 69 and 77%, respectively. However, this reduction was not statistically significant, but concurs with similar findings by Percival and Boyle (2009), who evaluated Nu-Film-P (similar to Nu-Film-17) and Designer (similar to Break-Thru S240) finding a 46 to 55% reduction in germination of conidia, as well as 60 to 63% fewer conidia forming compared with the untreated control. In the case of Nu-Film-17, reduction in appressorium formation can possibly be ascribed to the adjuvant forming a film over the leaf surface and thus preventing physical contact required for formation of appressoria, as was also postulated by Percival and Boyle (2009). In the case of Entreé (Hess and Foy, 2000), disruption might be attributed to adjuvant physical effects on the wax layer. These effects will have to be studied further.

CuOCl reduced conidium adhesion and viability, as well as germ tube length, indicating that CuOCl present on the leaf surface is an excellent attachment and germination inhibitor of *A. alternata* at the registered rate evaluated (2 g L^{-1}). Sprays with CuOCl alone and together with adjuvants also reduced appressoria formation and can be ascribed to the fungicidal effect of CuOCl. In a macroscopic study evaluating ABS lesion development on young leaves of Dancy tangerines, Mondal *et al.* (2007) reported 90 to 100% ABS disease control following sprays with copper hydroxide, while van Zyl *et al.* (2013) reported 50 and 75% control of *A. alternata* on young mandarin leaves at 0.34 and 0.68× of the current CuOCl registered concentration (2 g L^{-1}).

No significant synergistic effect was observed in our *in vivo* study when adjuvants were sprayed with CuOCl. It has to be noted, however, that adjuvants caused conidium and germ tube stress similar to that realised by CuOCl alone, indicating some direct influence on the pathogen.

To evaluate any direct effects of adjuvants alone, or possible synergistic effects together with copper oxychloride in an *in vitro* study, a microtiter assay using the oxidation and reduction of resazurin redox die (RZ) was used as described and previously used by Vega *et al.* (2012). Optimisation of the protocol for use with *A. alternata* was done for conidium concentration and incubation time but not for selected media since this was already done previously by Vega *et al.* (2012). Little difference was observed between RZ concentrations. The biggest influence on reduction of RZ was found to be conidial concentration and incubation time. As can be expected, higher conidial concentrations and longer incubation times resulted in higher RZ reduction. The highest percent and most stable (optimal) RZ reduction was found with 30 µM RZ and 1×10^5 conidia mL^{-1} over 24 hours incubation time. RZ was reduced over time from a dark blue colour to a variation of fluorescent bright pink over the 24 h. Longer incubation times led to bleaching of wells (total discolouration). As explained in Vega *et al.* (2012), this is caused by a second redox reaction step, followed by a reduction in RZ fluorescence from the final pink colour to colourless hydroresorufin. Our findings concurred to those by Vega *et al.* (2012). Aseptic loading of wells and sterile incubation techniques are very important since any contamination would cause bleaching of wells. The water only control wells that was not inoculated with spore suspension served as excellent indicators of contamination. If any colour change occurred in these control wells, contamination of the plate was confirmed and the trial had to be repeated.

The microtiter assay proved to be very successful in determining the EC_{50} an EC_{95} of CuOCl for *A. alternata*, and proved to be a superior technique to conventional alternatives. Measuring mycelial growth and count germinating conidia on media (*in vivo*) (Russel, 2004) was attempted in the present study on potato dextrose agar media amended with copper oxychloride to determine EC values. This methodology was used in a pilot trial to evaluate the

sensitivity of *A. alternata* to adjuvants alone and together with copper oxychloride, but proved to be unsuccessful due to copper oxychloride colouring traditional media a blue to green colour that prohibited the visualisation of germinating spores on the surface. In more specialised media (complete media as described by Bennett and Lasure (1991), CuOCl migrated out of solution to the bottom of the agar plates having little effect on *A. alternata* development.

The microplate protocol proved to be much more efficient, with loading of plates with various fungicide concentrations being possible. Loading with a multi-pipet was quick, aseptic and easy. Microplate reading took 12 min per plate, depending on the number of wells loaded. The effectiveness of copper oxychloride to inhibit germination was shown in the present study. Similar results were found by Rampersad (2011) with copper-based fungicides having little suppressive effect on mycelium growth of *Verticillium dahlia* on amended media plates but found good spore germination inhibition using a similar microplate protocol. Copper-based fungicides are mainly spore germination inhibitors due to their mode of action (McCallan, 1949; Richardson, 1997; RED, 2009). Copper oxychloride affects spore germination by suppressing oxygen uptake and therefore ATP synthesis during the respiration process (Richardson, 1997) and will therefore have little to moderate suppressive effect on mycelial growth on the surface of amended media. Rampersad (2011) filter sterilised stock solutions of fungicides with a 0.22 µm filter prior to amendment of culture media. This would have removed most copper particles from the stock solution since milled copper oxychloride particles have a mean diameter of 3.067 µm, depending on manufacturer (Schutte *et al*., 2012). Thus, it is questionable if evaluation of copper oxychloride sensitivity was accurate. Furthermore, copper-based fungicides need to be in solution to have an effect. This is not the case on the surface of agar media plates.

EC_{50} an EC_{95} values for CuOCl of *A. alternata* were calculated to be 35.50 mg L^{-1} and 281.66 mg L^{-1} CuOCl, respectively, using a Michaelis-Menten growth function. No comparable results could be found on the fungitoxic effect of CuOCl on conidium germination as most papers focussed on fungistatic effect on mycelial growth. Hassan *et al*. (2014), Ghazanfar *et al*. (2016) and Stepanovic *et al*. (2015) evaluated the sensitivity of *Alernaria solani* (causal agent of early blight of tomato) to various conventional fungicides through a fungicide amended media study. Hassan *et al*. (2014) found a concentration of 1000 mg kg^{-1} CuOCl to inhibit fungal growth by 62.23% after 7 days. Ghazanfar *et al*. (2016) found the inhibition of fungal growth to CuOCl to be 42% at 300 mg kg^{-1}. Conversely, Stepanovic *et al*. (2015) described relatively low EC_{50} values of *A. solani* for CuOCl at 13.27 to 15.63 mg L^{-1}. These studies indicate a fungistatic rather than fungitoxic effect of CuOCl on mycelial growth. Comparing results, the use of the RZ microplate protocol is more sensitive since inhibition was observed at lower Cu residues except for values found by Stepanovic *et al*. (2015). Interestingly, van Zyl *et al*. (2013) found 50% (68.44 mg kg^{-1}) and 95% (180.64 mg kg^{-1}) *A.*

alternata control on mandarin leaves after 24 h (based on Cu residues on sprayed leaves). Variation in sensitivity data found between the different studies can possibly be ascribed to variations in methodology used. This complicates direct comparison of sensitivity results. Comparing the EC_{50} and EC_{95} values with Cu residues achieved in chapter 5 might explain the phenomena where the addition of adjuvants to CuOCl sprays improved or realised similar control of ABS as the CuOCl alone spray, despite the observations that some of these adjuvants resulted in poorer spray deposition parameters and reduced copper residues on leaf surfaces. Cu residues realised in chapter 5 ranged from 57.02 mg kg^{-1} to 201.73 mg kg^{-1} thus possibly being present at a level where pathogen response was not dose dependent i.e too high too evaluate sublte pathogen response.

In vitro evaluation of adjuvants indicated no significant effect on conidium germination and germ tube growth or any significant synergistic effects with copper oxychloride at the concentrations used. None of the adjuvants active ingredients evaluated has any known fungistatic or fungitoxic action. Nita *et al*. (2007) evaluated a refined paraffinic oil (similar to Citrole100) and other adjuvants alone and together with various fungicides at recommended application rates for the control of *Phomopsis viticola* on grapevine leaves *in vitro* and found no fungicidal effect of adjuvants on the growth of the pathogen. Applied together with various fungicides, of which one was mancozeb, a contact fungicide, also did not improve disease control compared with fungicide alone applications.

In conclusion, no significant adjuvant-alone treatment effect was observed on *A. alternata* development on the surface of young mandarin leaves, neither was any direct or synergistic effect of adjuvants on conidium germination and growth observed in the *in vitro* study. CuOCl was shown to be a successful fungicide inhibiting conidium attachment and germination on the leaf surface, as well as germination and growth inhibition in the *in vitro* assay. Adjuvants induced pathogen stress and reduced appressorium formation and should be investigated further. Although informative, the findings in this study could not explain the anomalous results found in chapter 5. These results can possibly be ascribed to the effects of adjuvants on deposition quality of CuOCl sprays and future studies should focus on developing methodology to support histopathology studies on sensitive leaf surfaces, as well as development of more sensitive method of measuring deposition quality, especially on a microscopic scale.

REFERENCES

Abbott, C, P. The effect of adjuvants on apple disease management. Master thesis. Open access Theses. 739. http://docs.lib.purdue.edu/open_access_theses/739. 56 pp

Agrios, G.N. 2005. Stages in development of disease: The disease Cycle. Pages 80 – 81 in: Plant Pathology 5th Addition. Department of Plant Pathology, University of Florida. Elsevier Academic press.

Albrigo, L.G., Timmer, L.W., Townsend, K., Beck, H.W. 1997. Copper fungicides – Residues for disease control and potential spray burn. Proceedings of the Florida State Horticultural Society 110: 67-70.

Albrigo, L.G., Beck, H.W., Timmer, L.W., Stover, E. 2005. Development and testing of a recommendation system to schedule copper sprays for citrus disease control. Journal of ASTM International 2: 2-12.

Allen, E.A., Hoch, H.C., Stavely, J.R. 1991. Uniformity among races of *Uromyces appendiculatus* in response to topographic signalling for appressorium formation. Phytopathology 81: 883-887.

Baker, A.B., Procopiou, J., Hunt, M.H., 1975. The cuticles of Citrus species. Composition of leaf and fruit waxes. Journal of the Science of Food and Agriculture 26: 1093-1101.

Bargel, H., Cerman, Z., Koch, K., Netnhuts, C., 2006. Structure-function relationships of the plant cuticle and cuticular waxes – a smart material? Functional Plant Biology 33: 893-910.

Bennett, J.W., and Lasure, L.L. 1991. Growth media. Pages 441-458 in: More Gene Manipulations in Fungi. J. Bennett and L. Lasure, eds. Academic Press Inc., San Diego, CA.

Chang, Y.C., Séguin-Swartz, G. 2002. A rapid method for assessing the viability of fungal spores. Canadian Journal of Plant Pathology 24: 230-232.

Craver, T.L.W., Gurr, S.J., 2006. Filamentous fungi on plant surfaces. In: Riederer, M., Műller, C., (Eds.), Biology of the plant cuticle. Blackwell Pub. Annual Review of Plant Biology 23: 306-392.

Dean, R.A. 1997. Signal pathways and appressorium morphogenesis. Annual Review of Phytopathology 35: 211-234.

Ensikat, H.J., Ditsche-Kuru, P., Barthlott, W. 2010. Scanning electron microscopy of plant surfaces: simple but sophisticated methods for preparation and examination. In: A. Méndez-Vilas and J. Diaz (Eds.), Microscopy: Science, technology, applications and educations 248-255.

Epstein, L., Nicholson, R.L. 1997. Adhesion of spores and hyphae to plant surfaces. Pages 11-25 in: The Mycota, volume 5. Springer-Verlag, Berlin, Germany.

Gent, D.H., Schwartz, H.F., Nissen, S.J., 2003. Effect of commercial adjuvants on vegetable crop fungicide coverage, absorbtion and efficacy. Plant Disease 87: 591-597.

Ghazanfar, M.U., Raza, W., Ahmed, S.K., Qamar, J., Haider, N., Rasheed, M.H. 2016. Evaluation of different fungicides against *Alternaria solani* (Ellis and Martin) Sorauer cause of early blight of tomato under laboratory conditions. International Journal of Zoology Studies 5: 8-12.

Grayson, B.T., Webb, J.D., Batten, D.M., Edwards, D. 1996a. Effect of adjuvants on the therapeutic activity of dimethomorph in controlling vine downy mildew. I. Survey of adjuvant types. Pesticide Science 46: 199-206.

Grayson, B.T., Boyd, S.L., Walter, D. 1996b. Effect of adjuvants on the therapeutic activity of dimethomorph in controlling vine downy mildew. II. Adjuvant mixtures, outdoor-hardened vines and one-pack formulations. Pesticide Science 46: 207-213.

Hall, J.H., Holloway, P.J., Stock, D. 1998. Factors affecting foliar retention of some model adjuvant oil-in-water emulsions. Pesticide Science; Extended Summaries: 9th Pesticide Chemistry Congress: 320-322.

Hess, F.D., Foy, C.L. Interaction of surfactants with plant cuticles. Weed Technology 14: 807-813.

Hassan, M., Farooq, M., Gul, F. 2014. In vitro evaluation of some fungicides against common fungal pathogen of early blight and fruit rot of tomatoes. Journal of Applied Environmental and Biological Sciences 98: 424-429.

Kalina, M., Palmer, J.M. 1968. The reduction of tetrazolium salts by plant mitochondria. Histochemie 14: 366-374.

Kirkwood, R.C. 1999. Recent developments in our understanding of the plant cuticle as a barrier to foliar uptake of pesticides. Pesticide Science 55: 69-77.

Knoche, M., Lenz, F., Noga, G., 1992. Surfactant-induced phytotoxicity: evidence for interaction with epicuticular wax fine structure. Crop Protection 11: 51-56.

Knogge, W. 1998. Fungal Pathogenicity. Current Opinion in Plant Pathology 1: 324-32.

Lahoz, E., Tarantino, P., Mormile, P., Malinconico, M., Immirzi, B., Cermola, M., Carrieri, R. 2017. Evaluation of a new natural adjuvant obtained from locust bean gum to reduce the amount of copper necessary to control downy mildew of grapevine. Journal of Plant Disease Protection 125: 287-296.

McCallan, S.E.A. 1949. The nature of the fungicidal action of copper and sulphur. Botanical Review 15: 629-643.

Mendgen, K. 1996. Chapter 8: Fungal attachment and penetration. Pages 175 – 188 in: Plant Cuticles by G. Kerstiens. Oxford: BIOS.

Mondal, S.N., Bhatia, A., Shilts, T., Timmer, L.W. 2005. Baseline sensitivities of fungal pathogens of fruit and foliage of citrus to azoxystrobin, pyraclostrobin, and fenbuconazole. Plant Disease 89:1186-1194.

Mondal, S.N., Vicent, A., Reis, R.F., Timmer, L.W. 2007. Efficacy of Pre- and postinoculation application of fungicides to expanding young citrus leaves fot control of melanose, scab and alternaria brown spot. Plant Disease 91: 1600-1606.

Njombolwana, N.S., Erasmus, A., van Zyl, J.G., du Plooy, W., Cronje, P.J.R., Fourie, P.H. 2013. Effect of citrus wax coating and brush type on imazalil residue loading, green mold control and fruit quality retention of sweet oranges. Postharvest Biology and Technology 86: 362-371.

Nita, M., Ellis, M.A., Wilson, L.L., Madden, L.V. 2007. Evaluation of the curative and protectant activity of fungicides and fungicide-adjuvant mixtures on Phomopsis cane and leaf spot of grape: A controlled environment study. Crop Protection 26:1377-1384.

Peacock, J., van Rensburg, L., Krüger, H., van der Merwe, C.F. 1998. Liquid substitution: An alternative procedure for leaf surface studies with scanning electron microscopy. Scanning Microscopy 12: 401-412.

Percival, G.C., Boyle, S. 2009. Evaluation of film forming polymers to control apple scab (*Venturia inaequalis* (Cooke) G. Wint.) under laboratory and field conditions. Crop Protection 28: 30-35.

Rampersad, S.N. 2011. A rapid colorimetric microtiter bioassay to evaluate fungicide sensitivity among Verticillium dahlia isolates. Plant Disease 95: 248-255.

RED. 2009. Reregistration eligibility decision for coppers; U.S Environmental Protection Agency, Office of prevention, toxic substances, Office of pesticide programs, US government printing office: Washington, DC.

Rogiers, S.Y., Whitelaw-Weckert, M., Radovanonic-Tesic, M., Greer, L.A, White, R.G., Steel, C.C., 2005. Effects of spray adjuvants on grape (*Vitis vinifera*) berry microflora, epicuticular wax and susceptibility to infection by *Botrytis cinerea*. Australas. Plant Pathology 34: 221-228.

Richardson, W.H. 1997. Handbook of copper compounds and applications. CRC Press, Boca Raton, Florida.

Russel, P.E. 2004. Sensitivity baselines in fungicide resistance research and management. FRAC Monograph No. 3. Croplife International, Brussels.

Santier, S., Chamel, A. 1996. Penetration of triolein and methyl oleate through isolated plant cuticles and their effect on penetration of [^{14}C] quizalofop-ethyl and [^{14}C] fenoxaprop-ethyl. Weed Résistance 36:167-174.

Schutte, G.C., Kotze, C., van Zyl, J.G., Fourie, P.H. 2012. Assessment of retention and persistence of copper fungicides on orange fruit and leaves using fluorometry and copper residue analysis. Crop Protection 42: 1-9.

Solel, Z., Kimchi, M. 1998. Histo pathology of infection of Minneola tangelo by *Alternaria alternata* pv. *citri* and the effect of host environmental factors and lesion development. Journal of Phytopathology 78: 440-443.

Solel, Z., Timmer, L.W., Kimchi, M. 1996. Iprodione resistance of Alternaria alternate pv. Citri from Minneola tangelo in Israel and Florida. Plant Disease 80: 291-293.

Stepanović, M., Jevremović, S., Rekanović, E., Mihajlović, M., Milijaševic-Marćić, S., Potočnik, I., Todorović, B. 2015. *In vitro* sensitivity of *Alternaria solani* to conventional fungicides and a biofungicide based on tea tree essential oil. Journal of Pesticides and Phytomedicine 30: 25-33.

To, W.K., Fothergill, A.W., Rinaldi, M.G. 1995. Comparative evaluation of macrodilution of alamar colormetric microdilution broth methods for antifungal susceptibility testing of yeast isolates. Journal of Clinical Microbiology 33:2660-2664.

Tucker, S.L., Talbot, N.J., 2001. Surface attachment and pre-penetration stage development by plant pathogenic fungi. Annual Review of Phytopathology 39: 385-417.

Van Zyl, J.G., Fourie, P.H., Schutte, C.G., 2013. Spray deposition assessment and benchmarks for control of Alternaria brown spot on mandarin leaves with copper oxychloride. Crop Protection 46: 80-87.

van Zyl, S.A., Brink, J.C., Calitz, F.J., Coertze, S. and Fourie, P.H. 2010(a). The use of adjuvants to improve spray deposition and *Botrytis cinerea* control on Chardonnay grapevine leaves. Crop Protection 29: 58-67.

van Zyl, S.A., Brink, J.C., Calitz, F.J. and Fourie, P.H. 2010(b). Effects of adjuvants on deposition efficiency of fenhexamid sprays to Chardonnay grapevine foliage. Crop Protection 29: 843-852.

Vega, B., Liberti, D., Harmon, P.F., Dewdney, M. M. 2012. A rapid resazurin-based microtiter assay to evaluate QoI sensitivity for *Alternaria alternata* isolates and their molecular characterization. Plant Disease 96: 1262-1270.

Vicent, A., Armengol, J., García-Jiménez, J. 2007. Rain fastness and persistence of fungicides for control of Alternaria brown spot of citrus. Plant Disease 91: 393-399.

Wagner, P., Fűrstner, R., Barthlott, W., Neinhuis, C., 2003. Quantitative assessment to the structural basis of water repellency in natural and technical surfaces. Journal of Experimental Botany 54: 1295-1303.

Whiteside, J.O., 1976. A newly recorded Alternaria-induced brown spot disease on Dancy tangerines in Florida. Plant Disease Reporter 60: 326-329.

Whiteside, J.O. 1977. Sites of action of fungicides in the control of citrus melanose. Phytopathology 67: 1067-1072.

Wynn, W.K. 1981. Tropic and toxic responses of pathogens to plants. Annual Review of Phytopathology 19:237-255.

Yao, C., Myung, K., Wang, K., Johnson, A. 2014. Spray retention of crop protection agrochemicals on the plant surface. Chapter one in: Retention, uptake and translocation of agrochemicals in plants. ACS symposium series; American Chemical Society: Washington, DC, 2014.

Zabkiewicz, J.A. 2007. Spray formulation efficacy-holistic and futuristic perspectives. Crop Protection 26: 312-319.

Table 1. Percent adhered conidia (%), total germ tube length (µm), percent viable conidia (%), percent conidia or germ tube stress (%) measured on leaf disks treated with selected adjuvants alone and together with copper oxychloride.

Treatment	Adhered conidia (%)[a]	Total germ tube length (µm)[a]	Viable conidia (%)[a]	Conidia / germ tube stress (%)[a]	Appressoria formed[ab]
Control	83.50 a	90.56 ab	92.45 a	65.17 b	1.61 a
CuOCl	41.95 c	21.10 c	32.84 b	88.41 ab	0.06 a
Entreé	82.90 a	117.08 a	97.15 a	94.70 a	0.36 a
Break-Thru S240	91.27 a	101.98 ab	95.71 a	85.27 ab	1.26 a
Nu-Film-17	84.20 a	78.16 b	99.17 a	72.83 ab	0.49 a
Entreé + CuOCl	37.81 c	13.11 c	46.93 b	93.81 a	0.09 a
Break-Thru S240 + CuOCl	53.19 bc	22.05 c	31.99 b	93.54 ab	0.08 a
Nu-Film-17 + CuOCl	61.02 b	16.62 c	41.78 b	91.09 ab	0.03 a

[a]For each parameter separately, values in each column followed by the same letter do not differ significantly (P > 0.05) according to Tukey's HSD test.

[b]Average number of appressoria formed per germinated conidia.

Table 2. Mean pH and *Alternaria alternata* germination/growth inhibition (%) realised in microplate test wells amended with or without 35.50 (mg L^{-1}) of copper oxychloride and adjuvants, calculated from resazurin reduction as determined after 24h incubation with a microtiter multiplate reader.

Treatment[a]	pH[b]	Germination/Growth Inhibition (%)[b]
Control	6.36 e	0.00 b
CuOCl	6.20 h	49.14 a
Entrée	6.65 a	4.57 b
Break-Thru S240	6.39 d	0.00 b
Nu-Film-17	6.37 de	4.61 b
Citrole100	6.27 h	1.54 b
Villa51	6.28 g	1.63 b
Wetcit	6.52 g	2.91 b
Entrée + CuOCl	6.55 b	53.31 a
Break-Thru S240 + CuOCl	6.32 f	54.08 a
Nu-Film-17 + CuOCl	6.39 d	59.60 a
Citrole100 + CuOCl	6.21 g	52.92 a
Wetcit + CuOCl	6.27 g	58.60 a
Villa51 + CuOCl	6.30 f	53.71 a

[a]Adjuvant concentrations relative to copper oxychloride (2 g L^{-1}): Break-Thru S240 (1.5%); Nu-Film-17 (25%); Citrole 100 (150%); Villa51 (9%); Entrée (30%); Wetcit (50%). Treatment concentrations was calculated through the formula: adjuvant % × 0.8875 mL (relative to EC_{50} of CuOCl) in 250 mL complete media.

[b]Values followed by the same letter do not differ significantly ($P > 0.05$) according to Tukey's HSD test.

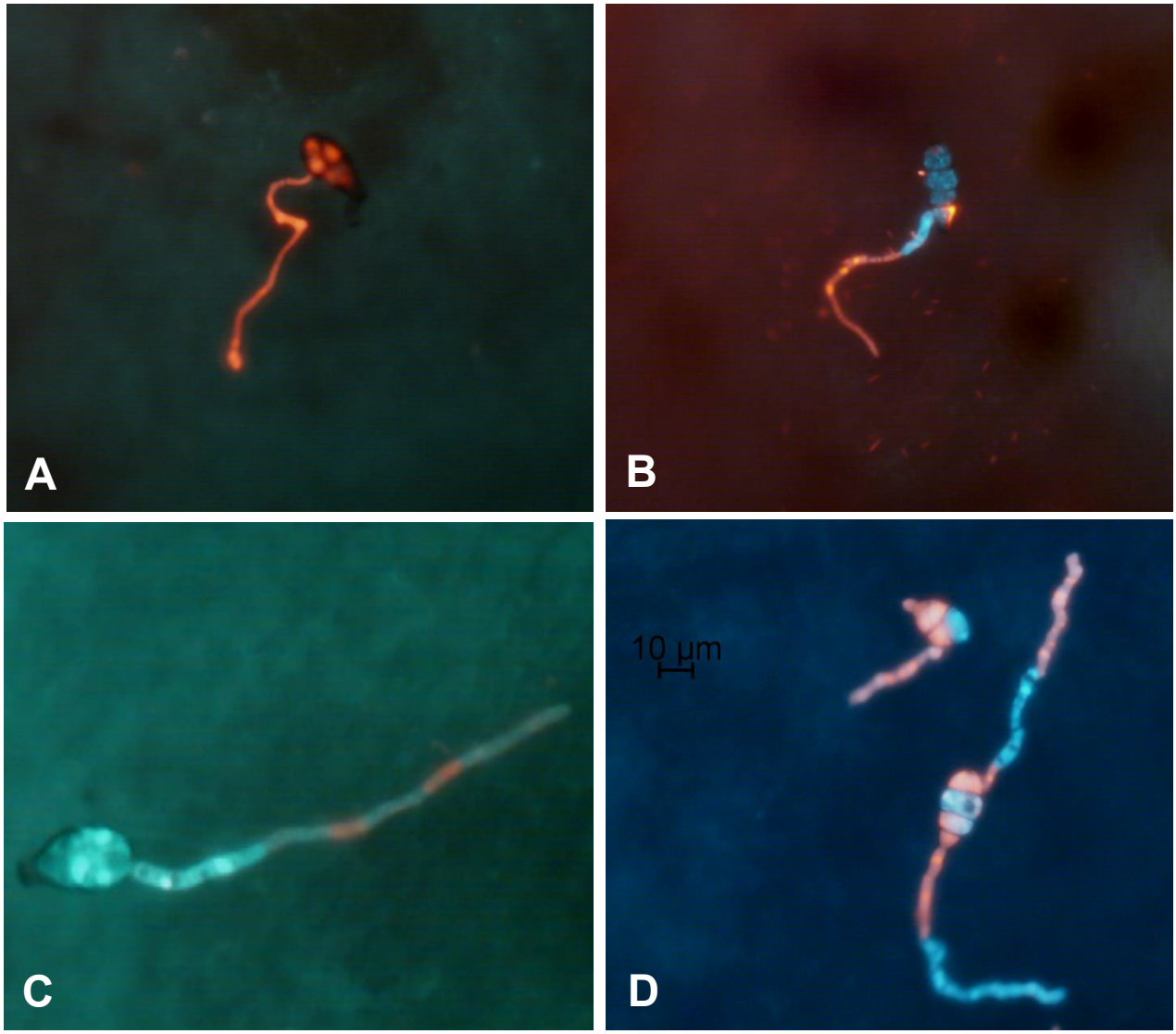

Figure 1. Germinating *Alternaria alternata* conidia stained with LIVE/DEAD BacLight staining solution after 6 h incubation on upper young mandarin leaf surfaces indicating various levels of stress due to influence of adjuvant and/or copper oxychloride. A: 100% stress; B: 50% stress; C: 20% stress; D: 90% stress (top conidia) and 40% stress (bottom conidia).

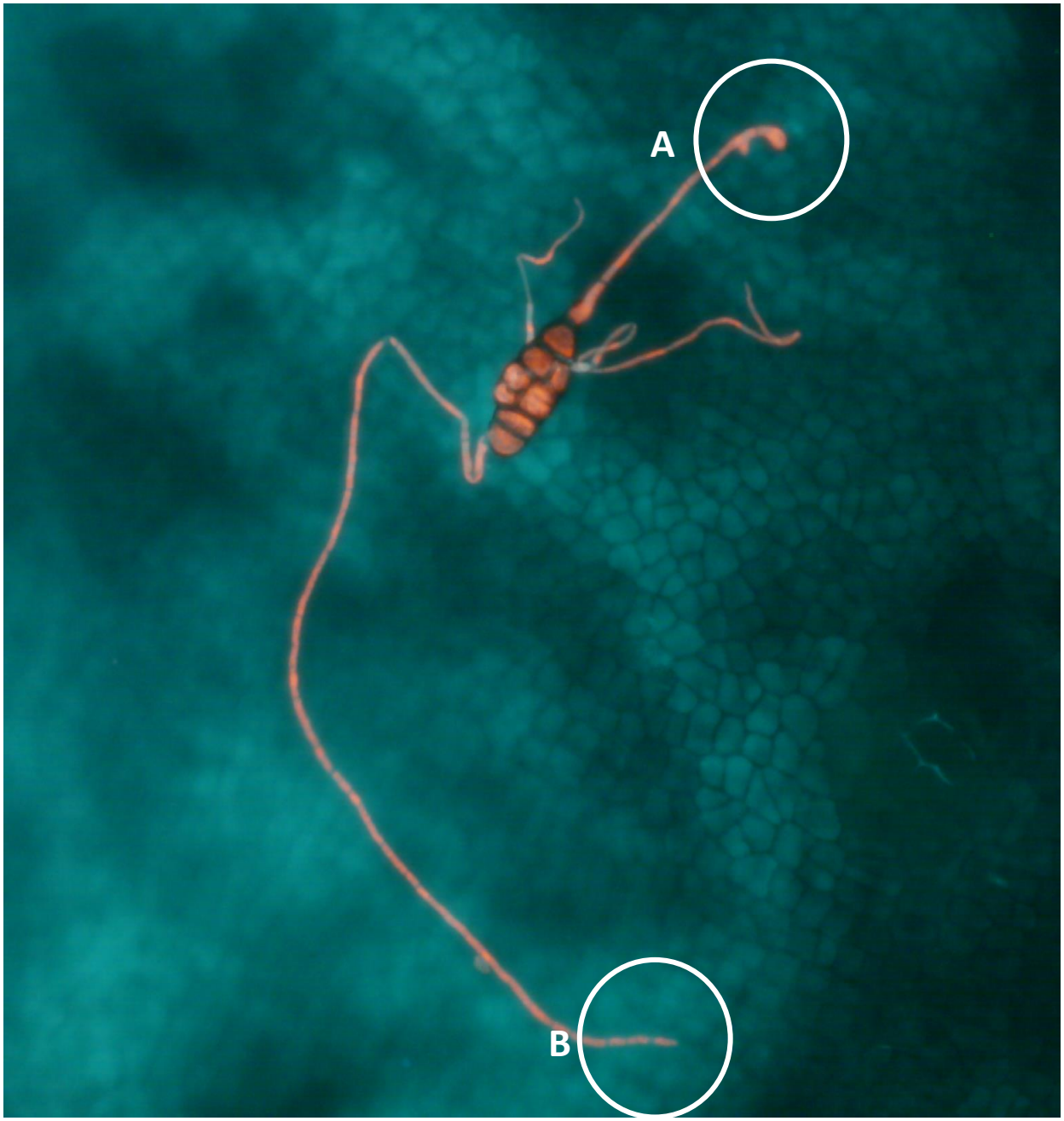

Figure 2. Germinating *Alternaria alternata* conidium stained with LIVE/DEAD BacLight staining solution after 6 h incubation on an upper young mandarin leaf surface with germ tube (A) forming and appressorium and (B) with normal germ tube growth.

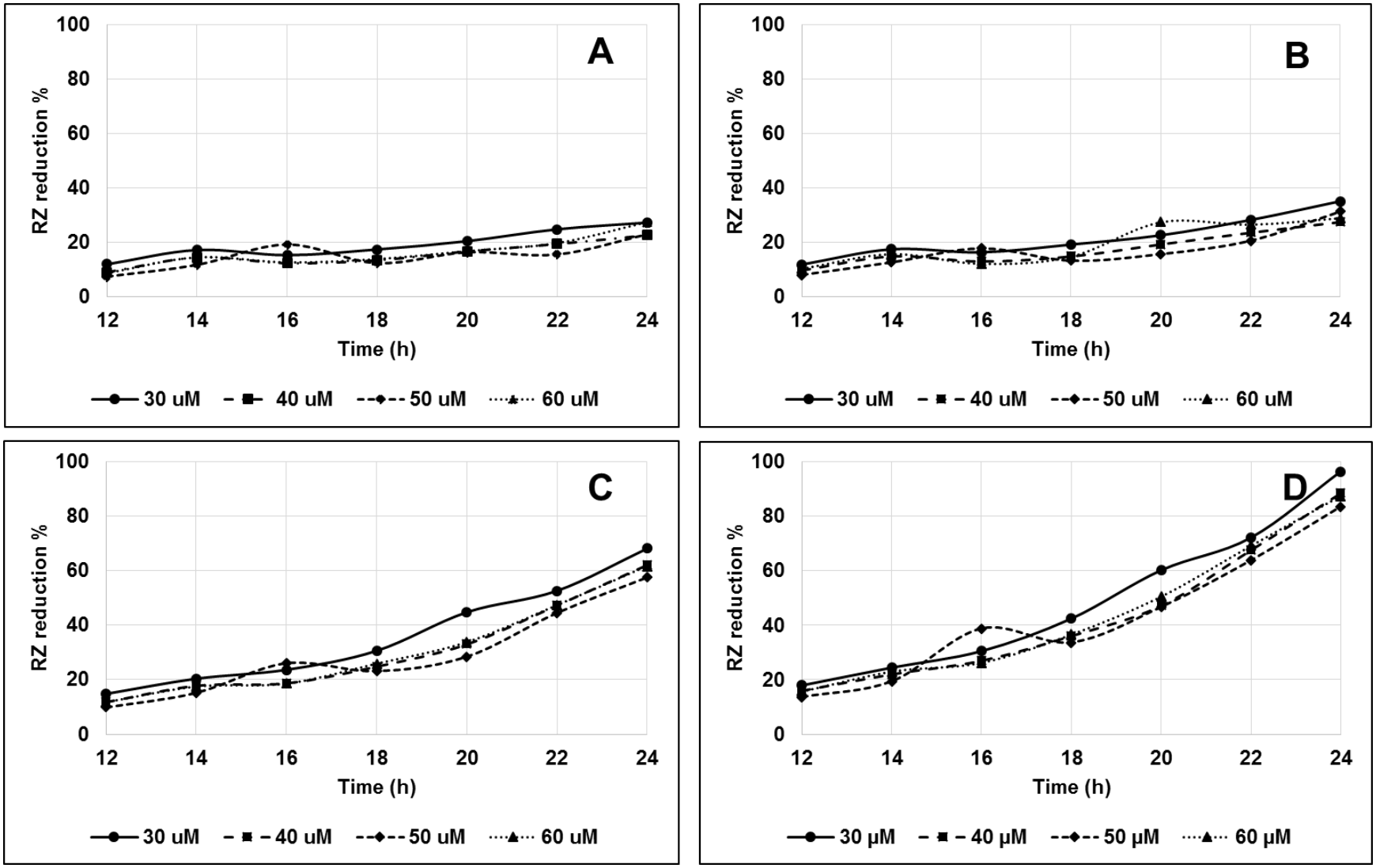

Figure 3. Resazurin (RZ) reduction (%) realised by different *Alternaria alternata* conidial concentrations (A: 5×10^3; B: 1×10^4; C: 5×10^4; D: 1×10^5 conidia mL^{-1}) using 30, 40, 50 and 60 µM RZ, measured at 12 to 24h incubation.

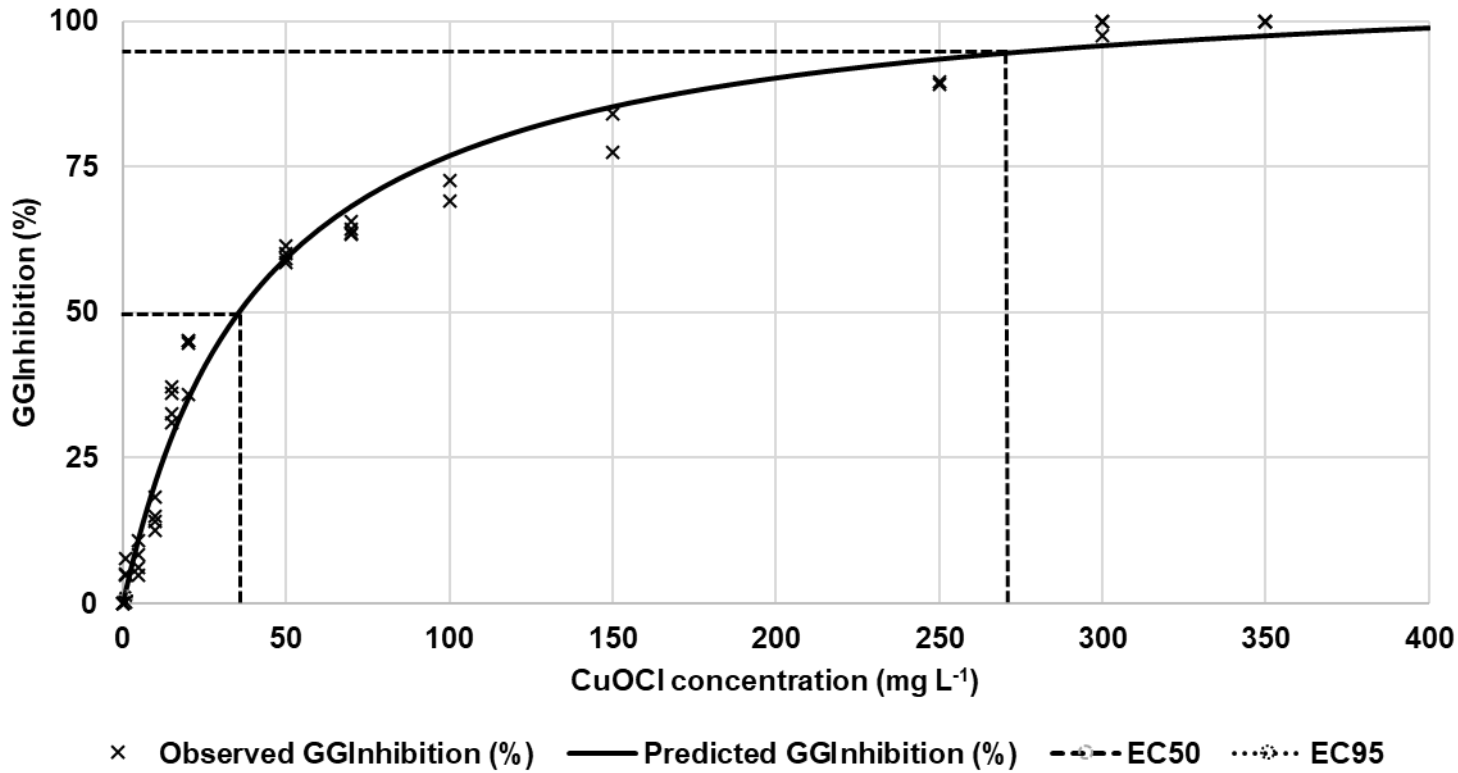

Figure 4. Mean *Alternaria alternata* germination/growth inhibition observed (%) and predicted germination/growth inhibition (%) realised in microplate test wells amended with different concentrations of copper oxychloride (mg L^{-1}), calculated from resazurin reduction as determined after 24 h incubation with a microtiter multi plate reader. A Michaelis-Menten growth function [germination/growth inhibition (%) = (109.164 × CuOCl concentration) / (41.9947 +CuOCl concentration)] was fitted to the data (R^2 = 0.774) and the CuOCl concentration resulting in 50% inhibition (EC_{50}) was calculated as 35.50 mg L^{-1} and 95% inhibition (EC_{95}) as 281.66 mg L^{-1} CuOCl.

CONCLUSION

South Africa is the second largest exporter of fresh citrus in the world. Producing disease and pest free citrus fruit will always be needed to ensure exports, especially in relation to phytosanitary pests like citrus black spot (*Guignardia citricarpa*) and false codling moth [*Thaumatotibia (Cryptophlebia) leucotreta*]. Effective disease control is dependent on effective deposition of the correct fungicide or pesticide on the susceptible target surface. Adjuvants have the potential to improve fungicide deposition and/or uptake, and through it, improve disease control. However, no research exists in this field in relation to fungicide spray deposition in citrus.

This study set out to evaluate the influence of adjuvants on spray deposition parameters. To achieve this, the objectives of this study was firstly to develop a spray deposition assessment protocol and deposition benchmarks indicative of biologically effective deposition quantities. Secondly, the deposition assessment protocol was used to evaluate the influence of adjuvants on deposition parameters in South African citrus orchards on different citrus types and cultivars and spray volumes used, and the effect it has on control of *Alternaria alternata*, the causal agent of Alternaria brown spot of mandarins, with copper oxychloride (CuOCl). A third objective was added to determine if adjuvants have any direct or indirect effect on *A. alternata* development and if there were any synergistic effects between evaluated adjuvants and CuOCl.

This study describes an improvement on a previously described spray deposition assessment protocols and provides new information on the suitability of a fluorescent pigment that has been used (Chapter 2). The deposition assessment protocol can be used to evaluate spray deposition parameters quantity, quality and uniformity in citrus and in other fruit tree crops. The protocol has been successfully used to evaluate spray machines, spray methodology (for example spray volume, tractor speed, canopy density etc.) in citrus, apples, tomatoes and blueberries in other studies.

By using control of Alternaria brown spot (ABS) of mandarins with copper oxychloride (Cu) as a model system, this study modeled fluorescent pigment deposition benchmarks indicative of effective disease control. These benchmarks are important in the biological interpretation of spray deposition. Fungicide dosage can also be evaluated leading to implementation of effective but environmentally sound application rates (Chapter 2).

The deposition assessment protocol and deposition benchmarks were used to evaluate two organosilicone adjuvants (Break-Thru S240 and Break-Thru Union) at different spray volumes in spray friendly and dense citrus canopies. The importance of canopy management was highlighted from the findings in this study. Citrus canopies, being large and dense, complicates spray penetration and therefore deposition on interior targets. Tree canopies

need to be well managed by pruning spray windows to improve spray penetration and through it deposition parameters on inner canopy targets. The detrimental effect of two organosilicone adjuvants Break-Thru S240 and Break-Thru Union at higher spray volumes in dense canopies was shown, as they increased run-off and reduced spray penetration. At higher spray volumes, it is therefore better not to include spray adjuvants when spraying unpruned, dense canopies. On the other hand, these adjuvants at lower spray volumes (3700 to 4800 L ha^{-1}) improved deposition quantity, efficiency and uniformity on the inner and outer canopy leaves, provided that the canopy is less dense (Chapter 3).

Fourteen adjuvants are currently registered in South Africa for use in citrus spray application. These adjuvants are regularly prescribed by agrochemical consultants and used by producers without knowing the impact it has on deposition of fungicides and pesticides. Six adjuvants were evaluated in this study. Adjuvants generally improved deposition uniformity and deposition quality, but these benefits were significantly influenced by spray volume and the specific adjuvant treatment. In general, the best and more consistently performing adjuvants were Break-Thru and Nu-Film-17, followed by Citrole100, with performance of Entreé, Exit, Villa51 and Wetcit being inconsistent. Suboptimal and irregular performance by adjuvants were ascribed to high spray volumes used and/or too high adjuvant concentration, which led to increased levels of run-off and poor deposition parameters. Deposition efficiency results indicated that lower volume sprays with adjuvants were more efficient. From these results, it is the author's opinion that the use of adjuvants at high fungicide spray volumes currently used in citrus is unnecessary and not economical since no significant benefit was shown (Chapter 4).

As was found in Chapter 3 and 4, laboratory spray results obtained in Chapter 5 also indicated that adjuvants have the potential benefit to improve deposition parameters. However, it also indicated the negative effects adjuvants can have on deposition parameters and residue loading if used at post-run-off volumes and too high product concentrations. The potential improvements of deposition parameters and residue loading therefore again depend on the interaction between spray volume, product concentration and target surface characteristics. Despite the effect on deposition quantity and Cu residue retention, adjuvant treatments generally gave similar or improved ABS control on leaf surfaces. Even so, the negative effect of certain adjuvants on Cu residue loading on target surfaces should be of concern since it is unknown if the reduced deposition quantities would be sufficient to realise sustained protection over time, or until the next protectant spray is applied, especially taking into account rain wash-off, weathering, fruit/leaf expansion under field conditions (Chapter 5).

Chapter 6 describes the evaluation of the effect of adjuvants alone and together with CuOCl on the growth and development of *A. alternata* on the leaf surface and through an *in vitro* microplate study. No significant adjuvant-alone treatment effect was observed on

conidium attachment, viability and germ tube growth on the surface of leaves, neither was any direct or synergistic effect of adjuvants on conidium germination and growth observed in the microplate study. CuOCl was shown to be a successful fungicide inhibiting conidium attachment and germination on the leaf surface, as well as germination and growth inhibition in the *in vitro* assay. Adjuvants induced pathogen stress and reduced appressorium formation and should be investigated further (Chapter 6).

Although informative, the findings in this study could not explain the anomalous results found in chapter 5, *i.e.* that poorer deposition parameters did not result in poorer ABS control. These results can possibly be ascribed to the effects of adjuvants on deposition quality of CuOCl sprays and future studies should focus on developing methodology to support histopathology studies on sensitive leaf surfaces, as well as development of a more sensitive method of measuring deposition quality, especially on a microscopic scale (Chapter 6).

This study confirmed the potential of adjuvants to improve spray deposition in citrus trees. However, this was mostly not apparent at high spray volumes and in dense citrus canopies. Reduced volume sprays together with correct adjuvant use can result in better deposition parameters than that of current dilute, high volume spray applications. However, this needs to be evaluated in seasonal deposition and bio-efficacy trials. Also, the increase in plant protection product concentrations with reduced volume sprays also needs to be evaluated in terms of safe use through evaluation of maximum residue limit (MRL) and phytotoxicity evaluations.

The outcomes of this study provide researchers, growers and consultants with the necessary tools and knowledge to evaluate spray application in future studies, and information to improve recommendation of adjuvant use in citrus spray programmes.